FRESHWATER FISH BIOGEOGRAPHY IN THE BERING GLACIER REGION, ALASKA

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By

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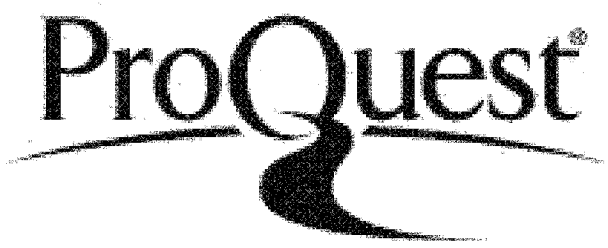

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By

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Abstract

Bering Glacier, Alaska, is Earth's largest surging glacier, with surges occurring approximately every 20–30 years since 1900. Surges and subsequent retreats lead to a dynamic environment for aquatic communities, as glacial ice over-rides landscapes and new habitats form during glacial retreat. Lands around Bering Glacier are administered by the State of Alaska and the U.S. Bureau of Land Management (BLM). Purposes of this study are to characterize fish communities and provide information relevant to their management for BLM. Given Bering Glacier's remoteness, little information exists regarding its fish communities.

Fish were collected over five summer field seasons (2002–2006), with 10 fish species collected in 80 lakes and streams. Results indicate that Dolly Varden (*Salvelinus malma*), threespine stickleback (*Gasterosteus aculeatus*), prickly sculpin (*Cottus asper*), and coho salmon (*Oncorhynchus kisutch*) are first to colonize new habitat after glacial retreat.

Ten locations with sympatric populations of anadromous and resident freshwater threespine stickleback were found. Geometric morphometrics and genetic analyses were conducted on these species pairs to test hypotheses regarding their formation. Possible origins include sympatric speciation, double invasion of anadromous fish, and independent colonization by resident freshwater fish from pre-existing lakes and streams along with anadromous fish.

Body shape analyses of anadromous vs. resident freshwater stickleback supported the independent colonization hypothesis, because of a lack of body shape co-variation

between groups, a lack of correlation of geometric morphometric variables with site age, and few fish with intermediate body shape at each site.

Origin hypotheses were tested by use of the frequency of the Euro-North American Clade (ENAC) vs. the Trans North Pacific Clade (TNPC) in the mtDNA as well as sequence divergence of a portion of the mtDNA gene NADH2. A greater proportion of TNPC fish exist in resident freshwater populations at all sites, supporting the independent colonization hypothesis. The NADH2 sequence data did not help to clarify the origins.

Future research at Bering Glacier should examine broader scale sequence divergence in genomes of stickleback and other colonizing fishes to advance the understanding of contemporary evolution and management implications in this newly formed aquatic landscape.

Dedication

This dissertation is dedicated to my father, Mark Weigner, who is always proud of me and always believes in me.

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Chapters two, three, and four have coauthors. Frank von Hippel is the coauthor on Chapter two and he contributed by providing research design, data collection and analyses, and manuscript review. Frank von Hippel is the coauthor on Chapter three and he contributed by reviewing and editing the manuscript. Nicole Nishimura, Frank von Hippel, and William A. Cresko are coauthors on Chapter four. N. Nishimura provided laboratory assistance. F. von Hippel reviewed and edited the manuscript. W.A. Cresko provided funding, research design, and aided with analyses.

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Chapter 1 General Introduction

1.1 Introduction

1.1.1 *The Bering Glacier System*

The Bering Glacier (60 – 61 N and 141 – 145 W) is the largest glacier in the world outside of Greenland and Antarctica. It originates in the Bagley Icefield (Fleisher, Cadwell, & Muller, 1998), drains approximately 5,200 km^2 of southcentral Alaska (Merrand & Hallet, 1996; Jaeger & Nittrouer, 1999), and is approximately 200 km long (Herzfeld & Mayer, 1997). It is bordered by the Gulf of Alaska and the Chugach and St. Elias mountains. At its Neoglacial maximum in approximately 1900, it terminated in an outwash plain just before the Gulf of Alaska (Calkins, Wiles, & Barclay, 2001). It is also the world's largest surging glacier (Post, 1969; Wiles et al., 1999).

A glacial surge is characterized by a short period of rapid advanceg, typically 1-2 years, followed by a longer period of recession that may last for a couple of decades (Post, 1969; Molnia & Post 1995; Josberger et al., 2006). During a surge, the terminus of the glacier overruns the glacial forelands and causes major changes to the ecosystem (Herzfeld & Mayer, 1997). Surges typically occur every 20-30 years (Molnia & Post, 1995; Muller & Fleisher, 1995; Calkin et al., 2001). Six surges occurred during the 20^{th} century; about 1900, about 1920, about 1938-1940, 1957-1960, 1965-1967, and 1993-1995 (Molnia & Post, 1995; Molnia, Post, & Carlson, 1996; Jaeger & Nittrouer, 1999). Surges typically punctuate extended periods of stagnation and/or recession (Molnia & Post, 1995).

The most recent surge, which occurred in 1993-1995, caused rapid advancing in the central region of the terminus of the Bering Lobe, including a 1500 meter advance in 17 days (Herzfeld & Mayer, 1997). Meanwhile, the terminus around the western side of Vitus Lake at Tashalich Arm experienced a retreat of 25 meters per day (Herzfeld & Mayer, 1997). The dynamics of surges cause different areas of the terminus to move at different rates throughout the surge (Herzfeld & Mayer, 1997), which has been the pattern for the past 1500 years (Molnia & Post, 1995). Therefore, different parts of the surrounding ecosystem are differentially disrupted. Surges are often accompanied by glacial outburst floods. These floods are capable of altering the environment as much as the surge itself. An outburst flood that occurred in Berg Lake during May 1994 released approximately 5,500,000,000 m^3 of water over 72 hours and lowered the water level by more than 50 m (Molnia & Post, 2010). A different outburst flood that occurred in Tsivat Lake during July 1994 carried ice chunks the size of a house 0.5 km from the terminus (Fleisher, Bailey, & Cadwell, 2003).

As the Bering Glacier flows out of the Bagley Icefield, it divides into the Bering Lobe and the Stellar Lobe. The Bering and Stellar Lobes terminate at a chain of proglacial lakes that are separated from the Gulf of Alaska by a narrow strip of land (Molnia & Post, 1995; Herzfeld & Mayer, 1997; Fleisher et al., 2003). The largest of these proglacial lakes is Vitus Lake, which is about 25 km long, 10 km wide and up to 150 m deep, though it is constantly changing (Brouwers & Forester, 1993; Molnia & Post, 1995; Josberger et al., 2006). The surface of Vitus Lake is approximately 2 m

above sea level (Brouwers & Forester, 1993) thus most of the lake lies below sea level. Since the end of the 1950s, Vitus Lake has drained through the Seal River into the Gulf of Alaska. The Seal River is 5 km long and 5-10 m deep; at high tide its surface is below sea level, as is the case with Vitus Lake (Merrand & Hallet, 1996; Molnia & Post, 1995). Before the 1950s, the Bering Glacier was drained by the Kaliakh, Tsivat, Tsiu, Midtimber, Seal, Tashalich, Kiklichk and Bering rivers (Molnia & Post, 1995). At present the Seal and the Kaliakh/Kosakuts rivers provide the primary drainage from the Bering lobe while the Bering River provides the primary drainage from the Stellar Lobe.

Before the 1950s, Midtimber Lake and Tsivat Lake (previously Tsivat River and is not the same as the proglacial Tsivat Lake) were proglacial rivers draining the Bering Lobe of the glacial. However, as the glacier began to recede, temporary hydrological channels dried up and the Seal River became the main drainage river. With the change in the drainage of the Bering Lobe to the Seal River only, Midtimber River, Tsivat River and Tashalich River evolved from proglacial rivers to lakes. Presently, Midtimber Lake and Tsivat River are open seasonally to the Gulf of Alaska during periods of increased precipitation and snow melt, while Tashalich Lake has a continually open but small outlet to the Gulf of Alaska (H.L. Weigner, pers. obs.). Tsiu River and Kiklichk River have evolved from proglacial to clear water rivers.

Glacial retreats in 1989 and again in 2006 caused outburst floods from Bentwood Lake into Vitus Lake via the north side of Bentwood Island, causing the water level in Tsivat, Tsiu, and Bentwood lakes to drop by approximately 14 m (H.L. Weigner, pers.

obs.). The outburst floods diverted the outlet from the Abandoned Channel and drainage from Bentwood, Tsiu, and Tsivat lakes now occurs around the north side of Bentwood Island into Vitus Lake. The Abandoned Channel is currently dry. The glacial surge of 1993-1995 advanced over the north end of Bentwood Island and closed the outlet to Vitus Lake, thus forcing the outlet to flow through the Abandoned Channel (Fleisher et al., 2003).

Proglacial Vitus Lake formed at the terminus of the Bering Lobe after the 1920 surge, and by 1938 it was 6 km long and 3 km wide (Molnia et al., 1991). Due to a warming climate, proglacial lakes around the Bering Glacier are growing at a rapid pace (Josberger et al., 2006).

Previous work on the fishes in the region consists of the Yakataga State Game Refuge Management Plan from 1999 by the Alaska Department of Fish and Game (ADF&G). Primary sampling for this management plan was conducted in the forelands of the Bering Glacier between the Gulf of Alaska and the current terminus. The terminus of the glacier has been receding from its Neoglacial maximum since approximately 1900, though some of the lakes and streams were overridden by the glacier as recently as the latest surge in 1993-1995 (Herzfeld & Mayer, 1997; Molnia & Post, 1995).

1.1.2 *Research Description and Organizing Hypotheses*

Appendix A, which was published in 2004 sets the framework for the study. It describes the youth of the freshwater environment and reports on the known threespine stickleback (*Gasterosteus aculeatus* species complex) species pairs at that time. The

paper discusses the morphological differences between the anadromous and resident freshwater threespine stickleback based on a suite a characters measured.

In chapter two, I discuss the relationships between fish species that occur in the region and environmental variables. The area sampled was a heterogeneous landscape of proglacial and periglacial oligotrophic lakes, glacial and clear melt-water streams, kettle ponds, wetlands, and tidally influenced lakes, streams and estuaries. There is no history of fish stocking in the area and humans have caused minimal disturbance. Chapter two also describes two atypical evolutionary pathways of freshwater fish in the region - species pairs of anadromous - resident freshwater threespine stickleback and populations of dwarf Dolly Varden (*Salvelinus malma*). Finally, I discuss management implications for the conservation of these unusual populations as well as the conservation of salmon habitat.

Seven types of species pairs of threespine stickleback with sympatric or parapatric ranges are known to naturally occur (McKinnon & Rundle 2002; Ólafsdóttir, Ritchie, & Snorrason, 2006). Of these, only the anadromous-resident freshwater species pair is located over a wide geographic area, having originated independently and repeatedly throughout the stickleback's range (McPhail, 1994). In the Bering Glacier region, 10 locations (six lakes and three streams) with anadromous – resident freshwater species pairs were identified. Vitus Lake contained two spatially separated locations with species pairs. In von Hippel and Weigner (2004; Appendix A), we proposed three hypotheses for the formation of these species pairs: sympatric speciation, double invasion by

anadromous fish from the Gulf of Alaska, or independent colonization from previously existing resident freshwater populations concurrent with colonization by anadromous stickleback from the Gulf of Alaska. In order to test these hypotheses, I employ geometric morphometrics (Chapter 3) and genetic analyses (Chapter 4).

In chapter three, I use geometric morphometrics to delve into differentiation of body shapes between sympatric populations of anadromous and resident freshwater threespine stickleback, and describe two additional species pairs not reported in von Hippel and Weigner (2004). I also examine the role of sexual dimorphism in body shape differentiation by analyzing anadromous males and females separately, as well as resident freshwater males and females. Due to the youth of the lakes and streams under study (less than 100 years in most cases), I expect to find body shape similarities between anadromous and resident freshwater members of a species pair if either sympatric speciation or double invasion has occurred. Conversely, if independent colonization has occurred, I expect differentiation of body shape due to a longer divergence time (possibly 1000s of years).

In chapter four, I analyze clade frequency of threespine stickleback based on the mitochondrial DNA (mtDNA) cytochrome b gene and sequence data from a portion of the NADH2 gene, also on the mtDNA. Threespine stickleback arise from one of two cytochrome b clades: the Euro-North American Clade (ENAC) or the Trans North Pacific Clade (TNPC). These clades formed approximately 2 million years ago when the Sea of Japan was isolated from the Pacific Ocean (Higuchi & Goto, 1996). Previous studies

showed that Alaska and British Columbia form a zone of admixture between the clades (Ortí et al., 1994; Deagle, Reimchen, & Levin, 1996; Thompson, Taylor, & McPhail, 1997; Cresko, 2000; Johnson & Taylor, 2004). Given the existence of the two clades within the region, if either sympatric speciation or double invasion occurred, I would expect to see similar clade frequencies within members of a species pair. However, if independent colonization occurred, I would expect to find different clade frequencies between members of a species pair. Because NADH2 is one of the most variable genes in the mitochondrial genome, it was chosen for sequencing. If either sympatric speciation or double invasion were the mode of formation of species pairs, then I would expect to find little sequence divergence between the anadromous and resident freshwater members of a species pair; however, if independent colonization was the mode of formation, then I would expect that resident freshwater populations would be more similar to each other than they would be to sympatric anadromous populations.

Chapter five provides a summary of each chapter's conclusions and identifies priority areas for future work.

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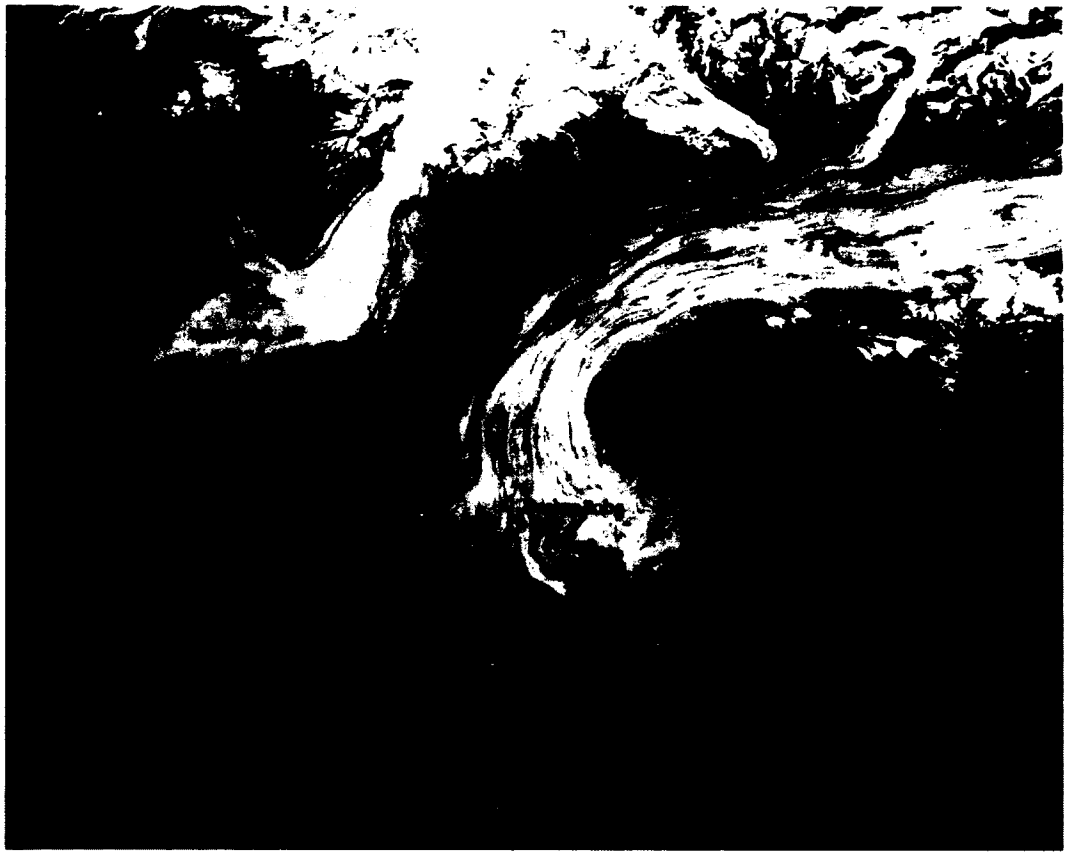

Figure 1.1.Bering Glacier Landsat imagery with key features discussed in the text identified.

Chapter 2 Biogeography and ecological succession in freshwater fish assemblages of the Bering Glacier region, Alaska[1]

2.1 Abstract

An inventory of fish species was conducted in the Bering Glacier region, Alaska, in 2002-2006. Ten species were collected: surf smelt (*Hypomesus pretiosus*), coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), Dolly Varden (*Oncorhynchus mykiss*), threespine stickleback (*Gasterosteus aculeatus*), prickly sculpin (*Cottus asper*), slimy sculpin (*Cottus cognatus*), Pacific staghorn sculpin (*Leptocottus armatus*) and starry flounder (*Platichthys stellatus*). All are either marine in origin or tolerant of salt water; consistent with this, fishes in the watershed tolerate a wide range of water qualities in fresh water. Stickleback, prickly sculpin, slimy sculpin, coho salmon, and Dolly Varden are found most commonly either because they are early colonizing species or they are able to out-compete early colonizers. Species that readily assume residence in fresh water were found equally often

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in isolated and connected lakes and streams, while diadromous and marine species were found primarily in lakes and streams with outlets. Except for Dolly Varden, species were more likely to be found in non-glacial than in glacial lakes and streams. Greater species richness was associated with the presence of aquatic vegetation and algae both of which provide structural complexity and indicate more abundant nutrient levels in otherwise oligotrophic waters. With the exception of Vitus Lake, which is tidally-influenced, fish species richness was low. Older lakes and streams support more species than younger aquatic habitats, presumably due to greater time for colonization and the formation of habitat complexity. This ever-changing aquatic landscape has been colonized by typical fish species in the region, but these colonists have evolved in atypical ways, including populations of dwarf Dolly Varden and stickleback species pairs.

2.2 Introduction

Understanding the biogeography of a region is paramount to population and community studies. Knowledge of the history of individual taxa allows us to anticipate the role each will exhibit in a new community [Wilson, 1961]. New lakes and streams formed in the wake of receding glaciers provide a diverse landscape for colonization and evolution of oceanic fishes in fresh water. These young, reticulated freshwater landscapes are subject to numerous disturbance factors. Habitat types include proglacial (ice contact at glacial terminus) and periglacial (ice contact at sides of glacier) lakes, kettle ponds, glacial and snow meltwater streams, oxbow lakes, tidally-influenced lakes and streams, and older, mature lakes, streams and estuaries. The combination of various

biotic and abiotic features of each type of freshwater system plays an important and complex role in the biogeography of fishes [Johnson et al., 1977; Dunham et al., 2003]. Freshwater biogeography of newly-deglaciated terrain is poorly understood. In Glacier Bay National Park, Alaska, Milner [1987; 1994; Milner and Bailey, 1989] investigated the colonization and development of freshwater stream communities, including colonization by salmonids, in relation to stream age and the temporal succession of aquatic macroinvertebrate communities. These studies focused on succession from newly-formed glacier meltwater streams to mature clear-water streams with a relatively stable hydrology. The biogeography of freshwater fishes in newly-deglaciated terrain with a complex system of streams, rivers, lakes, kettle ponds, and wetlands is less well understood.

Most biogeographic studies of freshwater fishes relative to glacial landscapes have been reconstructions of historical distributions during the Pleistocene glaciations based on geological evidence [McPhail and Lindsey, 1970; 1986; Lindsey, 1975; Lindsey and McPhail, 1986; McPhail 2007]. Currently, reconstructions of historic distributions are performed using phylogenetic analyses [e.g., Bernatchez and Wilson, 1998; Johnson and Taylor, 2004; Fraser and Bernatchez, 2005; Gagnon and Angers, 2006; Ray et al., 2006]. The on-going recession of the Bering Glacier gives us the opportunity to study fish colonization in real time in a complex, newly-formed freshwater landscape.

The purpose of this study is to document associations between fish species and abiotic and biotic factors in the Bering Glacier region and to document the distribution of

fish species in specific locations. We examine associations among fish species and their associations with substrate type, water appearance, water quality, aquatic vegetation and algae. We investigate which fishes are found in lakes versus streams, which colonize glacial lakes and streams, and which exist in isolated waters versus those connected to the Gulf of Alaska. We also discuss fish species richness in the context of the approximate year of a site's most recent deglaciation. We assemble all of the variables and build a predictive model to determine the occurrence of fish species based on these variables. By examining a suite of biotic and abiotic parameters, we investigate fish colonization and fish succession in aquatic habitats newly formed around the terminus of North America's largest glacier.

Finally, we report on the distribution of dwarf Dolly Varden (*Salvelinus malma*) and species pairs of threespine stickleback [*Gasterosteus aculeatus* species complex; von Hippel &Weigner, 2004] and discuss management implications.

2.2.1 *Study Area*

Bering Glacier (Fig. 2.1) is the largest glacier in the world outside of Greenland and Antarctica. It drains approximately 5,200 km^2 of southcentral Alaska [Merrand and Hallet, 1996; Jaeger and Nittrouer, 1999] and is approximately 200 km long [Herzfeld and Mayer, 1997]. It is the temperate zone's largest surging glacier [Wiles et al., 1999], with six surges reported since 1900 [Molnia et al., 1996; Jaeger and Nittrouer, 1999]. Surges typically occur every 20-30 years [Molnia and Post, 1995; Muller and Fleisher, 1995]. During a surge, the terminus of the glacier overruns the glacial forelands and

causes major changes to the young ecosystem [Herzfeld and Mayer, 1997]. The most recent surge, which occurred in 1993-1995, caused rapid advance in the central region of the terminus of the Bering Lobe, including a 1500 m advance in 17 days [Herzfeld and Mayer, 1997]. Meanwhile, the terminus around Tashalich Arm experienced a retreat of 25 m/d [Herzfeld and Mayer, 1997]. The dynamics of surges cause different areas of the terminus to move at different rates throughout the surge [Herzfeld and Mayer, 1997], which has been the pattern for the past 1500 years [Molnia and Post, 1995]. Therefore, different parts of the surrounding ecosystem are disturbed at different rates.

The terminuses of the Bering and Stellar Lobes form a chain of proglacial lakes separated from the Gulf of Alaska by a narrow strip of land [Fig. 2.1; Molnia and Post, 1995; Herzfeld and Mayer, 1997]. The largest of these proglacial lakes is Vitus Lake, which is about 25 km long, 10 km wide and up to 150 m deep, though it is constantly changing [Brouwers and Forester, 1993; Molnia and Post, 1995; Josberger et al., 2006]. Since the end of the 1950s, Vitus Lake has drained through the Seal River into the Gulf of Alaska. The Seal River is 5 km long and 5-10 m deep; at high tide its surface is below sea level [Molnia and Post, 1995; Merrand and Hallet, 1996]. Before the 1950s, Bering Glacier was drained simultaneously by the Tsivat, Tsiu, Midtimber, Seal, Tashalich, Kiklichk, Kosacuts, and Bering Rivers [Molnia and Post, 1995]. Now only the Bering, Kosacuts, and Seal Rivers provide primary drainage.

Most sampling was conducted in the forelands of the Bering Glacier between the Gulf of Alaska and the current terminus (Table 2.1, Fig. 2.1). The terminus of the glacier

has been receding from its Neoglacial maximum since approximately 1900 [Molnia and Post, 1995; Herzfeld and Mayer, 1997], though some of the lakes and streams were overridden by the glacier as recently as the latest surge in 1993-1995. The area sampled is a heterogeneous landscape of proglacial and periglacial oligotrophic lakes, glacial and clear meltwater streams, kettle ponds, and tidally influenced lakes, streams and wetlands. There is no history of fish stocking in the area and humans have caused minimal disturbance.

2.3 Methods

Fish were trapped using unbaited 1/8 inch mesh minnow traps, unbaited semi-oval traps, dip nets, and seine nets. Six to ten traps were set for approximately 24 hours at each site. Some species of fish may have been missed at some sites due to sampling methods that were biased towards small individuals located at the margins of aquatic habitat.

Fish were killed with an overdose of MS-222 anesthetic and preserved in 95% ETOH for DNA analysis or fixed in 10% buffered formalin and then preserved in 70% ETOH for morphological analysis. Fish were visually identified (when necessary under a Leica MZ6 dissecting microscope) using Morrow [1980], Pollard et al. [1997], and Mecklenburg et al. [2002]. Anadromous and resident freshwater threespine stickleback were distinguished from each other based on methods described in von Hippel and Weigner [2004]. Since Dolly Varden were not in breeding coloration, dwarfism was assumed based on small size and the appearance of dark parr marks coincident with the

presence of spots [Klemetsen and Grotnes, 1980; Parker and Johnson, 1991]. In normal-size Dolly Varden, parr marks disappear when the spots appear. Normal-size resident freshwater Dolly Varden are between 10-25 cm fork length [reviewed by McPhail, 2007].

Sampling was conducted from early May until early September, 2002 – 2006. We sampled for fish 221 times in 80 different lakes (including kettle ponds and former rivers that evolved into lakes) and streams. All traps where placed within 3 m of the shore and between 0.3-2 m deep. Field sites were reached by foot, ATV, helicopter and inflatable boat with an outboard engine. We did not sample bodies of water at random but on the basis of accessibility and previous reports from the Alaska Department of Fish and Game [1999].

Water quality measurements were made using a YSI-85 oxygen, conductivity, salinity and temperature meter and a Hanna pH meter. pH readings for 1 June - 2 July, 2004 were excluded from analysis due to a faulty meter. Surface water temperature was verified in the field using an alcohol thermometer. Salinity was verified in the field using a salinity refractometer. Latitude and longitude coordinates were collected using a Garmin Vista GPS, in decimal degrees and in datum WGS 1984.

Other observations, including substrate type, water appearance, presence of aquatic vegetation, and presence of macroscopic algae were made in the field, and isolation of lake or stream was recorded. Substrate type was divided into six categories: silt/clay/mud, sand (<0.25cm), gravel (0.25-5cm), cobble (5-25cm), boulders (>25cm), and bedrock. Water appearance was divided into five categories: clear, muddy/silty,

scummy/foamy, cloudy, or oily sheen; these categories were subsequently reduced to either clear or silty/not clear. Isolation is defined as lack of connection to the Gulf of Alaska by surface waters. Many sites sampled are seasonally connected based on rainfall and snowmelt. However, if they were not connected at the time of sampling, as determined visually from the air and walking the perimeter of the site, then they were classified as isolated, and these sites are likely to be isolated most of the time. Spawning salmon were visually identified by observation on the spawning grounds when possible.

Data were analyzed using SPSS version 11.5. Although some lakes and streams were sampled multiple times, each lake or stream is treated as a single observation in analyses. Chi-square analyses were used to test for significance of associations between pairs of fish species, occurrence in lakes versus streams, occurrence in isolated versus connected lakes and streams, occurrence in glacial versus non-glacial lakes and streams (glacial lake is defined as in glacial contact and glacial stream as fed by glacial meltwater), fish associations with aquatic vegetation, fish associations with algae, and fish associations with water appearance. Substrate type was not mutually exclusive and therefore could not be analyzed statistically. Associations for many fish species could not be analyzed statistically because of small sample sizes.

Approximate age of lakes and streams was determined from GIS layers that were built from aerial photographs and historical data from the region. GIS layers were constructed by ALTARUM. Approximate ages were divided into six categories based on when the lake or stream was deglaciated. Several of these sites were overrun by glacial

surges and the year of the most recent retreat of the glacier was considered as the year of formation. Data on the age of deglaciation were only available for lakes and streams in the Bering Glacier forelands. Lakes and streams on the perimeter were not included in the age analysis. If any part of the site sampled was not glaciated in 1900, the whole site was categorized as older than 1900. The categories were divided into before 1900, 1900-1967, 1968-1978, 1979-1981, 1982-1990, and after 1995. The categories were picked based on available GIS data. The 1993-1995 glacial surge covered all sampled sites that were formed after 1990, and hence there is no age category between 1991 and 1995; these sites formed again after the ice retreated subsequent to 1995. These age categories were then given age ranks from 1 (oldest) to 6 (youngest), and subjected to Spearman's correlation against rank of the number of fish species present in the site.

Binary logistic regressions were performed to build predictive models for the presence or absence of fish species based upon biotic and abiotic parameters. Continuous water quality variables were recoded and binned into groups to normalize their distributions. Salinity was divided into fresh water (0-0.5 ppt), brackish water (0.6-30.0 ppt), and marine water (>30.1 ppt). However, marine water was not present at any study site. Each variable was tested to determine if it alone had a significant effect on the presence or absence of a species. Non-significant terms and terms that were highly correlated with each other (>.80) were removed from the model. If a term needed to be removed due to a correlation with another term, the model was tested separately with each correlated variable to determine which had the best fit. Two-way interaction terms

were tested between the significant main effects [Landau and Everitt, 2004], but because none were significant they are not reported.

2.4 Results

Fish were captured in 79% (n = 63) of the 80 lakes and streams and in 90% of the 221 sampling trials. We collected ten fish species (Table 1), with resident freshwater threespine stickleback and prickly sculpin (*Cottus asper*) occurring in the greatest number of lakes and streams (n = 37 sites for each, 46% of all sites sampled). The resident freshwater stickleback was the most abundant species wherever it occurred. Surf smelt (*Hypomesus pretiosus*) were only collected in three locations in Vitus Lake, where they were collected dead on the beach. They are most likely found sporadically in the region. Coho salmon (*Oncorhynchus kisutch*) were found in 25 lakes and streams (31% of sites sampled). Sockeye salmon (*Oncorhynchus nerka*) were rarely observed in the region (just two streams). Fry were not trapped, though spawning adults were observed in both sites. Rainbow trout *(Oncorhynchus mykiss*) were also rarely found in the region (just four lakes and streams). Dolly Varden were trapped in 18 lakes and streams (23% of sites). Anadromous threespine stickleback were trapped in nine lakes and streams (11% of sites). All nine sites that contained anadromous stickleback also contained resident freshwater stickleback, giving rise to sympatric species pairs (Table 2.2). Slimy sculpin (*Cottus cognatus*) were captured in 14 water bodies (17.5% of sites). Pacific staghorn sculpin (*Leptocottus armatus*) and starry flounder (*Platichthys stellatus*) were captured in six sites (7.5% of sites).

2.4.1 *Associations between Fishes*

We examined associations between fish species found in sympatry. With ten species of fish and two varieties of stickleback, there are 55 possible pairwise associations, of which 47 were not encountered often enough to analyze statistically. Five of the eight association analyses were not significant (coho salmon and Dolly Varden, coho salmon and prickly sculpin, dolly varden and prickly sculpin, resident freshwater stickleback and slimy sculpin, and prickly sculpin and slimy sculpin). Resident freshwater stickleback have significant positive associations with coho salmon ($\chi^2 = 4.6$, df = 1, $p = 0.032$) and prickly sculpin ($\chi^2 = 19.8$, df = 1, $p < 0.001$), and a significant negative association with Dolly Varden ($\chi^2 = 8.2$, df = 1, $p = 0.004$).

Fish were captured alone at 19 sites. The species most commonly found alone was Dolly Varden. Resident freshwater species tended to occur sympatrically with few other fish species, while anadromous and marine species, in that order, were more often found coexisting with a larger number of other fish species (Fig. 2.2).

2.4.2 *Fish in Lakes or Streams*

The presence of fish was not significantly associated with whether a site was a lake (n=51) or stream (n = 29; $\chi^2 = 1.5$, df = 1, $p = 0.219$). However, some species were trapped more commonly in one habitat type than another. Coho salmon ($\chi^2 = 8.9$, df = 1, $p = 0.003$) and Dolly Varden ($\chi^2 = 17.3$, df = 1, $p < 0.001$) were trapped more often in streams, while sockeye salmon and rainbow trout were found only in streams (though the samples were too small to analyze statistically). Resident freshwater stickleback ($\chi^2 =$

4.2, df = 1, p = 0.040) and prickly sculpin (χ^2 = 8.9, df = 1, p = 0.003) were trapped more often in lakes. Slimy sculpin were trapped equally often in lakes and streams (χ^2 = 1.4, df = 1, p = 0.239). Remaining species were not found often enough to analyze statistically.

2.4.3 *Fish in Isolated Lakes and Streams*

Fish were trapped in 37 (71%) of 52 lakes and streams that were isolated from the Gulf of Alaska and in 26 (93%) of 28 lakes and streams connected to the Gulf of Alaska. Therefore, fish were more commonly found in connected lakes and streams (χ^2 = 5.1, df = 1, p = 0.024).

Although most species were found more frequently in connected lakes and streams, this trend was only significant for coho salmon (χ^2 = 26.9, df = 1, p < 0.001). Dolly Varden (χ^2 = 2.3, df = 1, p = 0.130), resident freshwater stickleback (χ^2 = 0.2, df = 1, p = 0.622), and prickly sculpin (χ^2 = 0.2, df = 1, p = 0.622) had no significant difference in the probability of being trapped in isolated or connected lakes and streams. Sample sizes were too small to analyze probabilities for the remaining species, though of these, only anadromous stickleback were found in isolated lakes and streams. For anadromous stickleback to occur in isolated sites, the sites must establish seasonal connections that were not evident at the time of sampling.

2.4.4 *Fish in Glacial Lakes and Streams*

Twenty-six (33%) of the lakes and streams sampled were glacial. Fish were found in 14 of them (54%) compared to 91% of non-glacial lakes and streams.

Therefore, we trapped fish in a higher proportion of non-glacial lakes and streams (χ^2 = 14.3, df = 1, $p < 0.001$).

With the exception of Dolly Varden, which were found more often in glacial lakes and streams ($\chi^2 = 8.7$, df = 1, $p = 0.003$), all species are found more commonly in non-glacial lakes and streams. Dolly Varden were trapped in 11 different glacial lakes and streams, or 42% of the total. Coho salmon were found in only three (12%) out of the 26 glacial lakes and streams trapped ($\chi^2 = 7.0$, df = 1, $p = 0.008$). Rainbow trout and sockeye salmon were not found in any glacial lakes or streams. Surf smelt, anadromous and resident freshwater stickleback, Pacific staghorn sculpin and starry flounder were all found in only one glacial lake, Vitus Lake. Resident freshwater stickleback were trapped significantly more often in non-glacial lakes and streams ($\chi^2 = 27.9$, df = 1, $p < 0.001$). Prickly sculpin are also seldom found in glacial lakes and streams compared to non-glacial lakes and streams ($\chi^2 = 14.8$, df = 1, $p < 0.001$). Remaining species were not captured often enough to be analyzed statistically.

2.4.5 *Fish Associations with Habitat Parameters*

Fish were more commonly found in lakes and streams with aquatic vegetation. Out of the 37 lakes and streams with aquatic vegetation, 35 (95%) also had fish present, while out of 43 lakes and streams without aquatic vegetation, only 28 (64%) also had fish present ($\chi^2 = 10.3$, df = 1, $p < 0.001$). Resident freshwater stickleback ($\chi^2 = 16.0$, df = 1, $p < 0.001$) and prickly sculpin ($\chi^2 = 12.6$, df = 1, $p < 0.001$) have statistically significant associations with the presence of aquatic vegetation. Remaining species were not

captured frequently enough to be analyzed statistically, except for Dolly Varden which showed no significant association.

Fish are also usually found in lakes and streams with algae. Out of the 49 lakes and streams with algae, 43 (88%) also had fish present, while out of 31 lakes and streams without algae, only 20 (65%) also had fish present ($\chi^2 = 6.1$, df = 1, p = 0.013). All fishes are found more often in lakes and streams with algae than without. There were significant associations between the presence of algae and coho salmon ($\chi^2 = 7.9$, df = 1, p = 0.005), resident freshwater stickleback ($\chi^2 = 6.0$, df = 1, p = 0.014), prickly sculpin ($\chi^2 = 4.0$, df = 1, p = 0.046), and slimy sculpin ($\chi^2 = 4.3$, df = 1, p = 0.039). Dolly Varden had a non-significant association with algae. Remaining species were not captured frequently enough to be analyzed statistically.

Substrate types were not mutually exclusive. Any combination of substrate could occur in a lake or stream, with the exception of bedrock, which was not present at any site. Substrate type did not appear to affect whether fish were present or absent, nor which species of fish were found at which sites (Table 2.3).

Fish were found more often in clear water than in muddy/silty, scummy/foamy, cloudy, or oily water (Table 2.4). When water appearance was categorized as clear or not clear, fish were found more often in clear water sites ($\chi^2 = 15.5$, df = 1, $p < 0.001$). Resident freshwater stickleback, however, were the only species to show a significant association with clear water ($\chi^2 = 10.1$, df = 1, $p < 0.001$). Coho salmon, Dolly Varden,

prickly sculpin, and slimy sculpin had no associations with clear water when analyzed statistically. Remaining species were not captured enough to analyze statistically.

2.4.6 *Water Quality*

Pacific staghorn sculpin, prickly sculpin, slimy sculpin, starry flounder, coho salmon, Dolly Varden, and anadromous and resident stickleback were collected sufficiently often to estimate water quality tolerances within the range of sites tested. Most fish species were trapped in a wide range of temperatures (Fig. 2.3a), salinities (Fig. 2.3b), pH values (Fig. 2.3c), conductivities (Fig. 2.3d), and dissolved oxygen values (Fig. 2.3e) that closely matched the distribution from all 221 sampling events. The species trapped most commonly in high salinities were Pacific staghorn sculpin, starry flounder, and slimy sculpin. The mean pH of water bodies in the region is 8.4, which is well above the pH of a typical freshwater habitat [Dodds, 2002].

2.4.7 *Approximate Age of Lakes and Streams*

Age of the lakes and streams was divided into six categories based on the timing of deglaciation. The older a lake or stream, the more likely it was to have fish and the more fish species we captured (Fig. 2.4). Currently, few fish species are found in sympatry; the mean number of fish species per lake or stream that deglaciated in 1978 or before is 3.2 while the mean number per lake or stream formed in 1981 or after is 1.4. Species found in the youngest sites were prickly sculpin (n = 3), slimy sculpin (n = 2), and resident freshwater stickleback (n = 2).

2.4.8 *Logistic Regression Models*

Presence of fish is best predicted by clear water (Wald = 8.37, p = 0.004), a non-glacial water body (Wald = 4.98, p = 0.026), and water bodies connected to the Gulf of Alaska (Wald = 5.65, p = 0.017). The presence of resident freshwater stickleback is best predicted by the presence of prickly sculpin (Wald = 9.02, p = 0.003), the presence of coho salmon (Wald = 5.68, p = 0.017), the absence of Dolly Varden (Wald = 8.87, p = 0.003), the presence of aquatic vegetation (Wald = 3.99, p = 0.046), and the presence of clear water (Wald = 7.81, p = 0.005). The presence of coho salmon is best predicted by water bodies connected to the Gulf of Alaska (Wald = 20.37, $p < 0.001$) and being found in non-glacial water bodies (Wald = 5.78, p = 0.016). The presence of Dolly Varden is best predicted by stream habitat (Wald = 11.22, p = 0.001) and a glacial water body (Wald = 6.77, p = 0.009). The presence of prickly sculpin is best predicted by lake habitat (Wald = 5.34, p = 0.021) and the presence of resident stickleback (Wald = 5.57, p = 0.018). The presence of slimy sculpin is best predicted by connection to the Gulf of Alaska (Wald = 8.44, p = 0.004).

2.4.9 *Distribution of Dwarf Dolly Varden*

Nearly all Dolly Varden trapped in the region appeared to be dwarfed (Table 2.5). Out of 18 different lakes and streams where Dolly Varden were found, nine are isolated (mostly Berg Lake area and Khitrov Lake area) and 11 are glacial.

2.5 Discussion

2.5.1 *Associations Between Fishes*

Fish communities are governed by predator - prey relations and competition for food and habitat [Evans et al., 1987]. Age and stability of the watershed are important for the maturation of fish communities from loose, random assemblages to more stable communities [Evans et al., 1987]. Freshwater habitats around the Bering Glacier are both young and unstable as they experience periodic glacial surges and glacial outburst floods. As the ice free landscape around the Bering Glacier continues to change and expand with deglaciation, more stable aquatic communities would be expected to form [Evans et al., 1987].

Most species were rarely found in the region (Table 2.1), presumably due to the youth of the landscape (most sites formed after 1900, and many formed in the last few decades; Fig. 2.4). However, we found a significant positive association between resident freshwater stickleback and coho salmon. Juvenile coho salmon prey on smaller threespine stickleback and their eggs [Reimchen, 1994]. A significant positive association also exists between resident freshwater threespine stickleback and prickly sculpin, most likely because adult prickly sculpin prey upon stickleback [Moodie, 1972a; McPhail, 2007; Messler et al., 2007] and they share many habitat requirements. A negative association was found between Dolly Varden and resident freshwater stickleback. Although Dolly Varden prey on stickleback [McPhail, 2007], the two

species are primarily found in different habitats. Dolly Varden were found in glacial streams whereas resident stickleback were found mostly in non-glacial lakes and streams.

2.5.2 *Fish in Isolated Lakes and Streams*

Four of the salmonid species were either found significantly more often in streams than lakes, or were found only in streams. In contrast, resident freshwater stickleback and prickly sculpin were found significantly more often in lakes. Lakes and streams can be isolated from the Gulf of Alaska or connected. Connection to the Gulf of Alaska is essential for anadromous life histories, such as those of most salmonids.

Since anadromous fish in the Bering Glacier region spawn in rapidly changing rivers and ephemeral streams, they may often become trapped in oxbow lakes, pools or former rivers that have become lakes. Some streams in the Bering Glacier Watershed are only connected once a year or every few years to the Gulf of Alaska, while others remain connected until the shifting ice front and outburst floods alter the hydrology sufficiently to change drainage patterns. Fish species that can assume a resident freshwater life history and reproduce in isolated freshwater habitat, such as Dolly Varden, threespine stickleback and prickly sculpin, readily adapt to the changing aquatic landscape. This likely explains why these species were found equally often in isolated and connected lakes and streams. However, coho salmon are strictly anadromous [Fry, 1973], and hence they were found significantly more often in connected than in isolated lakes and streams. Those spawned in isolated habitats may survive if the habitat is reconnected to the sea during times of high water, a pattern that is common in the region. However, if

an outlet to the sea fails to reconnect, these fish will retain the morphology of juveniles and fail to undergo sexual maturation [Fry, 1973].

Ephemeral hydrological networks provide unequal opportunities to different fish species to colonize different habitats, including isolated lakes and streams [Hershey et al., 2006]. Movements of the glacier alter hydrological channels and thus allow some fishes to colonize lakes that have become unavailable to others [Hershey et al., 2006]. As the stabilization of hydrological networks progresses with deglaciation, an increasing number of lakes and streams will likely become isolated due to the lack of seasonal connections from glacial meltwater and the establishment of regular stream channels that are no longer influenced by ice dams that alter drainages. This should further sort the species so that only those able to evolve into resident freshwater forms are found in isolated fresh waters.

2.5.3 *Fish in Glacial Lakes and Streams*

Glacial waters have low temperature, nutrient levels, and food resources, and high levels of suspended sediments [Gislason et al., 1999]. Waters with a high suspended sediment load present poor habitat for most fish species. Suspended sediments hinder visibility and thereby may interfere with reproduction and food acquisition. Sedimentation also leads to infilling of gravels and therefore poor oxygenation of fish embryos, such as those of salmonids, and developing young in the substrate. The lack of relationship between fish species and substrate size (Table 2.3) is consistent with findings in streams in Glacier Bay National Park, Alaska [Milner, 1987]. Some fish species, such

as salmon, require course substrates, such as large gravel and small cobbles for proper construction of their redds and development of their embryos. However, multiple substrate types were typically present at a site, and much of the sediment is course glacial outwash. As expected, fish were captured more often in clear water (Table 2.4), and fish are more likely to be found in non-glacial lakes and streams.

Dolly Varden were the only species that was more likely to be found in glacial lakes and streams. Previous studies in Glacier Bay National Park showed that Dolly Varden are able to thrive in the harsh environment of glacial streams and lakes because they require less habitat complexity than other fishes, such as coho salmon [Milner and Bailey, 1989]; furthermore, Dolly Varden are the first of the salmonids to colonize new stream habitat [Milner, 1994].

Surf smelt, anadromous and resident threespine stickleback, Pacific staghorn sculpin, and starry flounder were found in one glacial lake, Vitus Lake. Vitus Lake is unique within the study area because it is both large and tidally influenced, which likely facilitate its colonization by anadromous or marine fish and diversify the water chemistry to such a degree that many microhabitats are available in the lake, from fresh water at stream inlets to salinities up to 18 ppt at depths of 60 m [Josberger et al., 2006]. These factors likely explain the relatively high fish species richness in Vitus Lake (eight of ten fish species from the region, including both forms of threespine stickleback; Table 2.1). With the exception of Vitus Lake, fish species diversity in glacial lakes is typically low (Table 2.1)

2.5.4 *Fish Associations with Habitat Parameters*

Habitat complexity promotes fish community development by providing diversified niches with a richer prey base and refuge from predators. The Bering Glacier Watershed is mostly young without much structural complexity to its aquatic habitats. As expected, the waters with aquatic vegetation and algae (and therefore some structural complexity and niche variation) were most likely to contain fish. Species such as sculpins, which are ambush predators, tend to occur in complex habitats that provide cover [Wootton, 1998; McPhail, 2007]. Resident freshwater threespine stickleback with a benthic life history also tend to occur in shallow water with cover provided by aquatic vegetation and woody debris [Hagen 1967; Messler et al., 2007]. Furthermore, stickleback males prefer to build their nest where it is concealed by aquatic vegetation to minimize risk of predation and to increase the quality of parental care by reducing courtship intrusions, nest raids and territorial encounters [Hagen, 1967; Black & Wootton, 1970; Jenni, 1972; Moodie, 1972b; Kynard, 1978, 1979; Sargent & Gebler, 1980; Sargent, 1982; FitzGerald, 1983; Lachance & FitzGerald, 1992].

As the glacier continues to recede, the watershed will likely evolve from ice contact, unstable lakes and streams that are over-run by glacial surges and outburst floods every 20-30 years to mature lakes and streams with a relatively stable hydrology. This, in turn, should lead to a more complex habitat characterized by aquatic vegetation, algae, woody debris and a diversified prey base. Local fish species richness (alpha diversity) will likely increase as a consequence. Our results are consistent with this pattern. The

older a lake or stream, the more likely it is to have fish and the more fish species it has [Fig. 2.4]. In addition to having more time for habitat complexity to form, older lakes and streams have had more time for fish colonization. As lakes and streams age, their fish communities grow beyond the first few early colonizing species to include species more dependent on already established ecological interactions.

2.5.5 *Water Quality*

Fish can often tolerate brief changes in water quality by modifying their behavior. Long term changes to water quality, however, require adjustments to biochemical and physiological processes [Wootton, 1998].

A broad range of surface water temperatures was found in the region (Fig. 2.3a), which is consistent with a diversity of habitat types from massive proglacial lakes to small isolated ponds. Water temperature is one of the most important factors in determining the distribution of fishes due to its influence on dissolved oxygen and rates of physiological and biochemical processes [Wootton, 1998]. Deep lakes possess thermoclines, which a fish might need to swim through on a daily basis, and shallow water will often fluctuate several degrees over a 24 hour period [Wootton, 1998]. Not surprisingly, Bering Glacier fishes, most of which are early colonizing species and therefore adaptable to new environments, are found in a wide range of water temperatures characteristic of the habitat diversity (Fig.2.3a).

Fish species in the Bering Glacier region also have broad salinity tolerances (Fig. 2.3b), as is common in northern watersheds colonized after the melting of Pleistocene ice

sheets [Pielou, 1991]. This is expected since the newly deglaciated watershed was colonized by marine species and salt-tolerant anadromous and resident freshwater species that pioneer as colonists in newly-formed freshwater habitat.

Three marine species were collected (surf smelt, Pacific staghorn sculpin and starry flounder). Even though these species are classified as marine, they are also sometimes found in brackish or fresh water [Gunter, 1942; McAllister, 1963; Morrow, 1980]. Surf smelt were only found in Vitus Lake, which they reached by swimming up the Seal River, presumably with the incoming tide. Pacific staghorn sculpin and starry flounder are both found at the upper ends of tidally influenced lakes and streams [Gunter, 1942]. Prickly sculpin, which can assume either a resident freshwater or a catadromous life history [McAllister and Lindsey, 1961], are found in freshwater lakes and streams as well as in estuaries [Brown et al., 1995]. Taken together, our results indicate that resident freshwater fishes tend to be found in freshwater sites with few other fish species, while anadromous and marine fishes are found in freshwater sites with an intermediate and a high number of other fish species, respectively (Fig. 2.2). This may be because marine fishes can osmoregulate in lower salinities to a greater degree than freshwater fishes can osmoregulate in higher salinities [Gunter, 1942] leading to a more restricted habitat range for resident freshwater species.

Most lakes and streams sampled are less than about 100 years old and have a mean pH of 8.4, which is on the alkaline side of typical fresh water. Such elevated pH levels may be typical in glacially-influenced freshwater systems due to the effect of

glacial sediments. Similar levels are found in Glacier Bay National Park [Engstrom et al., 2000]. The tolerance of the fishes to elevated pH (Fig. 2.3c) is likely due to their marine origin. The normal range of pH for the marine environment is 7.5-8.5 [Knutzen, 1981], while the pH of fresh water usually ranges from 6.0 – 8.0 [Dodds, 2002]. Even within Vitus Lake the pH varies considerably. Josberger et al. [2006] found the pH in two vertical profiles in Vitus Lake to be between 5 and 6, though these profiles were collected well away from our sampling locations. They found the pH along a vertical profile in Berg Lake to be between 8.5 and 9, which is more consistent with our results

Conductivity levels varied widely in the region (Fig. 2.3d) due to the tidal influence from the Gulf of Alaska. Higher conductivity levels in fresh water indicate a greater availability of dissolved ions, which translates into lower physiological expense for fishes to acquire needed ions.

Dissolved oxygen is essential for proper functioning of fish [Wootton, 1998]. Salmonids are not tolerant of poorly oxygenated water, which results in greater mortality of eggs and alevins and poor growth of parr. Some other fishes are more tolerant of a wide range of dissolved oxygen [Wootton, 1998]. The dissolved oxygen concentration of some shallow water sites with fish were too low for sustained fish survival (Fig. 2.3e); presumably, fish swim out of these areas when their oxygen demand increases.

2.5.6 *Predictive Models*

Logistic regression models support inferences from bivariate comparisons. Presence of fish is best predicted by clear, non-glacial water that is connected to the Gulf

of Alaska. Models examining determinants of presence of individual fish species are consistent with their habitat preferences. For example, Dolly Varden presence is best predicted by a habitat being a glacial stream.

2.5.7 *Early Colonizing Species*

Remarkably, 79% of all lakes and streams sampled had at least one species of fish. This is undoubtedly an underestimate of the true frequency of lakes and streams with fish since our trapping efforts likely missed fish at some sites. These lakes and streams are colonized from the ocean by diadromous species or from glacial refugia by freshwater species. Therefore, the potential pool of colonist species is relatively small.

Early colonizing species are those found by themselves (unless these displaced the original colonists) and those found in the youngest sites. The two species most commonly found by themselves were Dolly Varden (n= 9 sites) and prickly sculpin (n =5 sites; Table 2.1). Species found in the youngest sites were prickly sculpin, slimy sculpin, and resident freshwater stickleback. If, as these data indicate, Dolly Varden, prickly sculpin, slimy sculpin and resident freshwater stickleback are early colonizing species, then they should often be found in sites with relatively few other fish species. This is the case, with the exception of slimy sculpin, which are found in sites with an intermediate number of other fish species (Fig. 2.2). Our data further suggest that early colonizing species of fish can be expected to be found in most lakes and streams in a deglaciating landscape, including newly-formed sites.

The most commonly occurring species in the Bering Glacier Watershed, resident freshwater stickleback and prickly sculpin, followed by coho salmon, Dolly Varden and slimy sculpin (Table 2.1), may have a competitive advantage over subsequent colonists by virtue of their adaptability and head start. Their colonization sets the stage for more complex ecological interactions involving fish as predators, prey, and competitors. Anadromous species that colonize newly formed lakes and streams (such as salmonids and stickleback) are also an important source of marine-derived nutrients to an oligotrophic system, which support the complexity of succession in both freshwater and surrounding riparian communities [Hicks et al., 2005]. Early colonizing anadromous fishes are therefore of key importance to the successional processes that facilitate colonization by other species.

2.5.8 *Established Communities*

Portions of the Bering Glacier region overlap with the Yakataga State Game Refuge. The major streams of the refuge were surveyed for fish species during the late 1990s [Alaska Department of Fish and Game, 1999]. The sampling events that occurred in streams outside of the Bering Glacier's influence during the 1990s give insight into what species may be present in the future, as the glacier continues to recede and the freshwater environment stabilizes.

2.5.9 *Stickleback Species Pairs*

Coexistence of reproductively isolated anadromous and resident freshwater sticklebacks in streams occurs frequently throughout the threespine stickleback's range

[McPhail, 1994; McKinnon and Rundle, 2002; Boughman 2007]. However, such pairs have only been identified in a few lakes: Lake Azabachije in Kamchatka [Ziuganov et al., 1987], Lake Sana in the Kuril Islands [discussed in Mori, 1990], lakes Harutori, Akkeshi and Hyotan on Hokkaido Island [Mori, 1990; Higuchi et al., 1996], Mud Lake [Karve et al., 2008] and a few nearby lakes in the Matanuska-Susitna Valley, Alaska [Bell, unpublished data], and coastal lakes formed subsequent to the Great Alaska Earthquake of 1964 on Middleton Island, Alaska [Gelmond, 2007]. In the Bering Glacier region we discovered three stream pairs and six lake pairs of anadromous and resident freshwater threespine stickleback species [Table 2.2; von Hippel and Weigner, 2004]. One of the species pairs is located in proglacial Vitus Lake, which is the first report of a stickleback species pair of any kind in a proglacial lake. The stickleback species pairs in the Bering Glacier Watershed, and particularly the Vitus Lake pair, represent an important component of the biodiversity of the Bering Glacier forelands.

2.5.10 *Dwarf Dolly Varden*

The populations of dwarf Dolly Varden in the Berg Lake and Khitrov Lake areas are isolated by massive waterfalls and glacial ice, respectively. Furthermore, these and other Dolly Varden populations are unusual, and deserve further study (Table 2.5). Little is known about their life history and population structure. It is not understood if these populations are dwarfed due to environmental constraints, such as poor nutrition, high density, and elevated pH (Table 2.5), and/or due to genetic factors, such as a mutation in the insulin like growth factor 1 allele or allele fixation due to the founder effect, genetic

drift, inbreeding, or selection. Dwarf populations of Dolly Varden also occur in the Matanuska-Susitna Valley [M. A. Bell, pers. comm.] and the Bristol Bay region [McPhail and Lindsey, 1970] of Alaska, but these locations deglaciated thousands of years ago, while many of the Bering Glacier sites are less than 100 years old.

Since the Berg Lake and Khitrov Lake fish live above dispersal barriers, a phylogenetic analysis may provide interesting insights into how Dolly Varden colonized such isolated habitat. Possibilities include delivery by piscivorous birds who bring live prey to their young [M. S. Christy, pers. comm.] or dispersal through glacial conduits. Such unusual methods of colonization would likely be reflected in low genetic diversity due to a population bottleneck. Phylogenetic relationships with surrounding populations may yield the source population(s) of the colonists. Furthermore, the question arises as to whether Dolly Varden became dwarfed in these young sites or if they colonized these sites while already dwarfed.

2.5.11 *Management Implications*

The stickleback species pair in Vitus Lake deserves protection as the only known species pair that exists in a proglacial lake anywhere in the world. The greatest threats to unusual and important stickleback populations in Alaska are exotic fishes (such as northern pike, *Esox lucius*), stocked salmonids, human impacts on water quality, water withdrawals for human use, and climate change leading to habitat loss [von Hippel, 2008]. None of these are current threats in Vitus Lake, but in the event that potentially damaging activities are proposed in the future, such as fish stocking, management

decisions should be informed by the presence of this unique species pair. Similarly, every effort should be made to prevent the introduction of exotic aquatic species, including fish. Invasive aquatic species would likely harm many of the freshwater fish species native to the Bering Glacier Watershed.

The last surge and outburst floods of 1993-1995 destroyed the salmon runs in Vitus Lake. Coho salmon have returned to Vitus Lake (Table 2.1), though the magnitude of the run is unknown. The Tsiu and Kiklichk Rivers remain popular coho salmon sport fishing rivers, and we trapped coho parr in both. We also found coho parr in the Tsivat and Kosakuts Rivers and Tashalich Lake, as well as a small lake adjacent to the Bering River and 19 other locations (Table 2.1). Adult sockeye salmon have been observed in the Tsiu and the East and West Forks of the Tsivat River (Table 2.1), and there have been reports of adult pink salmon (*Oncorhynchus gorbuscha*) in the Kiklichk River. It is not known if other runs of Pacific salmon are present in the Bering Glacier Watershed. A thorough sampling of the streams in late summer and fall should be performed to determine which salmon species are present and the strength of their runs. Sampling in the spring when salmon emerge from their redds would provide additional information on population dynamics. For example, pink and chum (*Oncorhynchus keta*) salmon go to sea immediately after emergence from redds [McPhail and Lindsey, 1970], and hence may have been missed in our sampling. The habitats of current and potential salmon streams need to be protected from pollution events or other causes of habitat degradation.

The dynamics of the Bering Glacier have created a complex of proglacial and periglacial lakes, kettle ponds, glacial and snow meltwater streams, oxbow lakes, tidally-influenced lakes and streams, and older, mature lakes, streams and estuaries. This ever-changing aquatic landscape has been colonized by typical fish species in the region, but some of these colonists have evolved in atypical ways. Berg Lake, which drains the Bering Glacier independently of Vitus Lake, experienced an outburst flood in 1994 that lowered its surface by more than 100 m in three days [Payne et al., 1997]. Berg Lake is also seemingly impossible to colonize due the massive waterfalls that drain the lake, and yet its small tributaries contain dwarf Dolly Varden. Dwarf Dolly Varden are also found in the isolated Khitrov Lake area, and stickleback species pairs are found in at least nine locations. As we learn more about the fishes of the Bering Glacier Watershed, we may find additional, unusual populations. It is clearly a watershed of paramount importance to understanding colonization and succession of aquatic organisms in newly deglaciated terrain. It warrants its status as a Research Natural Area with protections in place to safeguard the natural succession of its aquatic habitats.

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Table 2.1 Location of all sites sampled, number of times each site was sampled, and fishes found at each site

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Bentwood Lake	2	60.18	143.26											
Berg Lake Pond	1	60.4244	143.76715					X						
Berg Lake Stream 1	3	60.41	143.86					X						
Berg Lake Stream 2	1	60.42326	143.84619					X						
Berg Lake Stream 3	2	60.43	143.79					X						
Berg Lake Stream 4	6	60.427	143.67					X						
Berg Lake Stream 5	2	60.412	143.73					X						
Lake on Bering River	6	60.36	143.973		X				X	X	X	X		
Stream near Bering River	1	60.2756	143.97131		X			X			X			
Creek 1	12	60.103	143.36		X				X	X	X	X	X	X
Creek 2	9	60.09	143.35		X				X	X	X	X		X
Hana Lake	1	60.23716	143.12201								X			
Khitrov Lake	1	60.3951	143.47476					X						
Stream in Khitrov Hills	1	60.39976	143.48659					X						

Table 2.1 Continued

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Kiklichk River	3	60.017	143.803		X			X		X	X			
Kosakuts River	4	60.27	143.015		X			X				X		
Midtimber Lake	9	60.07	143.33						X	X	X	X		
Stream in Suckling Hills	1	60.0345	143.83661					X						
Stream below Suckling Hills	1	60.02073	143.82176		X		X	X						
Tashalich Lake	6	60.039	143.63		X				X	X			X	
Tsiu Lake	2	60.19115	143.20921								X	X		
Tsiu River	10	60.08	143.05		X	X		X	X	X				X
East Tsivat Lake	1	60.13512	142.97578							X				
Tsivat Lake	2	60.2315	143.13					X			X			
East Fork Tsivat River	1	60.13509	142.98289		X		X			X				
Tsivat River	3	60.065	143.25						X	X	X	X		
West Fork Tsivat River	1	60.16788	142.98744		X	X		X				X		
Unnamed Lake	5	60.102	143.55		X				X	X	X			

Table 2.1 Continued

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Stream into Vitus Lake	1	60.1498	143.26956		X			X			X	X		
Vitus Lake	59	60.1	143.3	X	X			X	X	X	X	X	X	X
Unnamed	1	60.08626	143.59593							X	X			
Unnamed	1	60.12875	143.5657								X			
Unnamed	2	60.0933	143.54							X			X	X
Unnamed	1	60.07311	143.44769										X	
Unnamed	1	60.07116	143.4408		X					X			X	X
Unnamed	3	60.11	143.475							X	X	X		
Unnamed	1	60.1161	143.47565							X	X			
Unnamed	1	60.11666	143.47559							X	X			
Unnamed	1	60.07656	143.39952							X	X			
Unnamed	1	60.17439	142.88318		X					X	X			
Unnamed	1	60.28817	143.27142											
Unnamed	1	60.28145	143.26598											

Table 2.1 Continued

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Unnamed	1	60.27804	143.27007											
Unnamed	1	60.2735	143.11859											
Unnamed	2	60.274	143.12											
Unnamed	2	60.23	143.12		X		X				X			
Unnamed	1	60.19212	143.20744											
Unnamed	1	60.17346	143.17477		X					X		X		
Unnamed	1	60.15723	143.21997				X			X		X		
Unnamed	2	60.1702	143.286		X							X		
Unnamed	1	60.15536	143.27312		X									
Unnamed	1	60.12015	143.27409								X			
Unnamed	1	60.1204	143.28148											
Unnamed	1	60.12083	143.27992											
Unnamed	2	60.11587	143.29259		X					X	X			
Unnamed	2	60.11794	143.30305		X					X	X			

Table 2.1 Continued

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Unnamed	2	60.1185	143.304		X					X	X			
Unnamed	2	60.1172	143.304							X	X			
Unnamed	1	60.11991	143.30804							X	X			
Unnamed	2	60.12018	143.303		X					X				
Unnamed	1	60.1217	143.30627											
Unnamed	1	60.12245	143.31848		X									
Unnamed	1	60.12247	143.32005											
Unnamed	1	60.11792	143.33102							X	X			
Unnamed	1	60.42302	143.8459											
Unnamed	1	60.14207	143.72112							X	X			
Unnamed	1	60.14292	143.658											
Unnamed	1	60.14577	143.6366								X			
Unnamed	1	60.1189	143.67191							X	X			
Unnamed	1	60.10201	143.6897							X	X			

Table 2.1 Continued

Site	Times Sampled	Location		Fish Species										
		Lat (N)	Long (W)	*Hp*	*Ok*	*On*	*Om*	*Sm*	*GaA*	*GaR*	*Ca*	*Cc*	*La*	*Ps*
Unnamed	1	60.10155	143.68843							X	X			
Unnamed	1	60.08538	143.59091							X	X			
Unnamed	1	60.455	144.2083							X				
Unnamed	1	60.27523	143.97087											
Unnamed	1	60.2760	143.97153								X			
Unnamed	1	60.39596	143.476											
Unnamed	1	60.39689	143.47847											
Unnamed	1	60.3978	143.47951											
Unnamed	1	60.06223	143.31793							X	X			
Unnamed	1	60.08607	143.59273							X	X			

Note: Site name is given if the lake or stream is named. Differences in the specificity of the GPS coordinates depend on the size of the lake or stream and the number of times sampled. *Hp* = surf smelt. *Ok* = coho salmon. *On* = sockeye salmon. *Om* = rainbow trout. *Sm* = Dolly Varden. *Ga*A = anadromous threespine stickleback. *Ga*R = resident freshwater threespine stickleback. *Ca* = prickly sculpin. *Cc* = slimy sculpin. *La* = Pacific staghorn sculpin. *Ps* = starry flounder.

Table 2.2. Locations, species pair category (lake or stream), and habitat details of the species pairs of anadromous and resident freshwater threespine stickleback

Location	Latitude (N)	Longitude (W)	Species Pair Category	Habitat Details
Bering River Lake Site	60.35771	143.97263	Lake	Small lake adjacent to Bering River
Creek 1	60.10482	143.35008	Stream	Stream draining into Vitus Lake
Creek 2	60.09770	143.35117	Stream	Stream draining into Vitus Lake
Midtimber Lake	60.08	143.35	Lake	Lake formerly a river
Tashalich Lake	60.03905	143.62566	Lake	Lake formerly a river
Tsiu River	60.08	143.06	Stream	River and wetlands
Tsivat River	60.065	143.247	Lake	Lake formerly a river
Unnamed Lake	60.10195	143. 55113	Lake	Small lake adjacent to Vitus Lake
Vitus Lake	60.1	143.3	Lake	Proglacial lake

Note: Differences in the specificity of the GPS coordinates depend on the size of the lake or stream and the number of times sampled.

Table 2.3. The presence and absence of fish by substrate type

	Substrate Type by Site (%)				
	Silt, Clay, Mud	Sand	Gravel	Cobble	Boulder
Fish Present	22.1	22.1	21.6	20.1	28.0
Fish Absent	17.5	22.8	22.8	21.1	15.8

Note: Substrate types are not mutually exclusive.

Table 2.4. The presence and absence of fish by water appearance

	Water Appearance by Site (%)				
	Clear	Muddy/Silty	Scummy/Foamy	Cloudy	Oily Sheen
Fish Present	68.8	22.1	5.2	2.6	1.3
Fish Absent	33.3	55.6	0	11.1	0

Note: Water appearance categories are mutually exclusive.

Table 2.5. Locations and habitat details of dwarf Dolly Varden sites

Location	Latitude (N)	Longitude (W)	Habitat Type	pH
Berg Lake area	60.41	143.86	Isolated glacial stream	8.65
Berg Lake area	60.42326	143.84619	Isolated glacial stream	8.20
Berg Lake area	60.43	143.79	Isolated glacial stream	7.90
Berg Lake area	60.427	143.67	Isolated glacial stream	9.58
Berg Lake area	60.42440	143.76715	Isolated glacial pond	6.50
Berg Lake area	60.412	143.73	Isolated glacial stream	7.35
Stream near Bering River	60.27560	143.97131	Non-glacial stream draining into glacial lake	-
Khitrov Lake	60.39510	143.47476	Isolated glacial lake	7.50
Khitrov Stream	60.39976	143.48659	Isolated glacial stream	9.10
Kiklichk River	60.017	143.803	Non-glacial river	7.75
Kosakuts River	60.27	143.015	Glacial river	8.09
Stream below Suckling Hills	60.02073	143.82176	Non-glacial stream	8.90
Stream in Suckling Hills	60.03450	143.83661	Isolated non-glacial stream	7.70
Tsiu River	60.08	143.05	Non-glacial river	8.38
Tsivat Lake	60.2315	143.13	Glacial lake	8.22
West Fork Tsivat River	60.16788	142.98744	Non-glacial stream	7.85
Stream into Vitus Lake	60.14980	143.26956	Non-glacial stream	6.60
Vitus Lake	60.1	143.3	Glacial lake	8.17

Notes: Differences in the specificity of the GPS coordinates depend on the size of the lake or stream and the number of times sampled. All populations appear to be dwarfed with the possible exception of the Dolly Varden in the Tsiu River. These fish were not trapped; however they were visually identified when angled and placement into the proper life history category was not made.

Figure 2.1. Landsat imagery of the Bering Glacier region showing all 80 sampling locations. Some locations were sampled more than once, but each location is only indicated once on the map. Landmarks named are discussed in the text. Image was captured September 10, 2001.

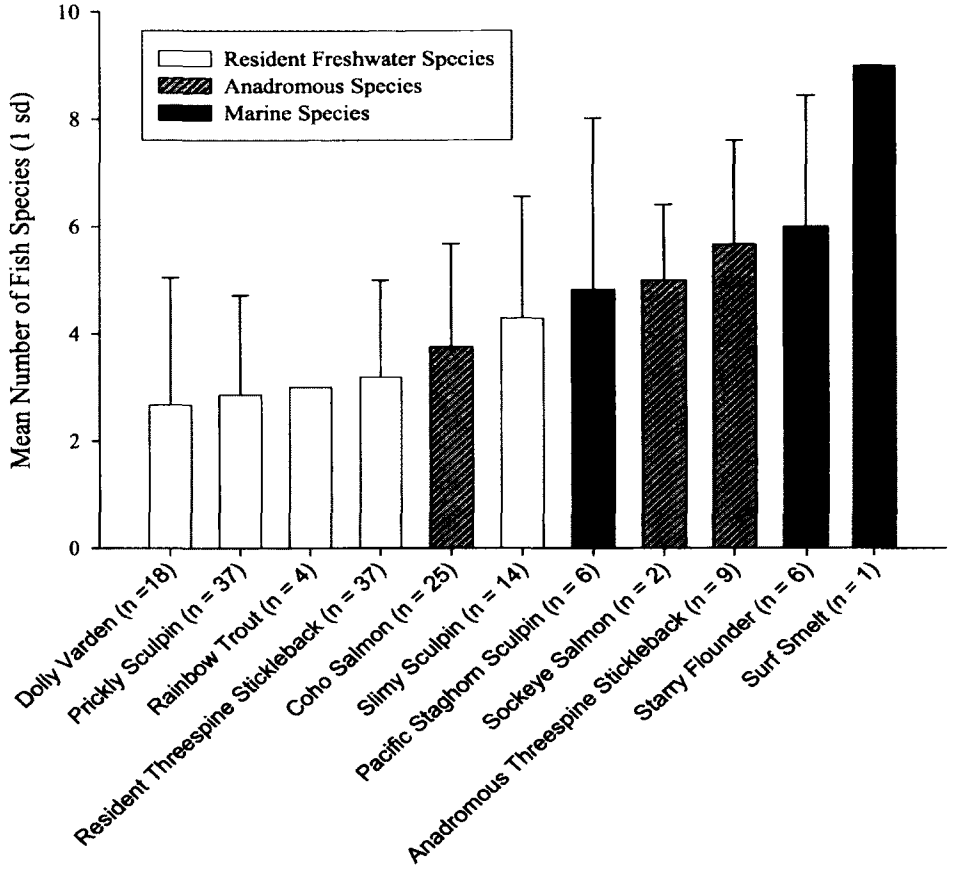

Figure 2.2. Mean number of fish species found with each of the ten species trapped in freshwater sites. Resident and anadromous threespine stickleback are counted as a single species in terms of number of species in a site. Prickly sculpin may adopt either a resident freshwater or catadromous life history.

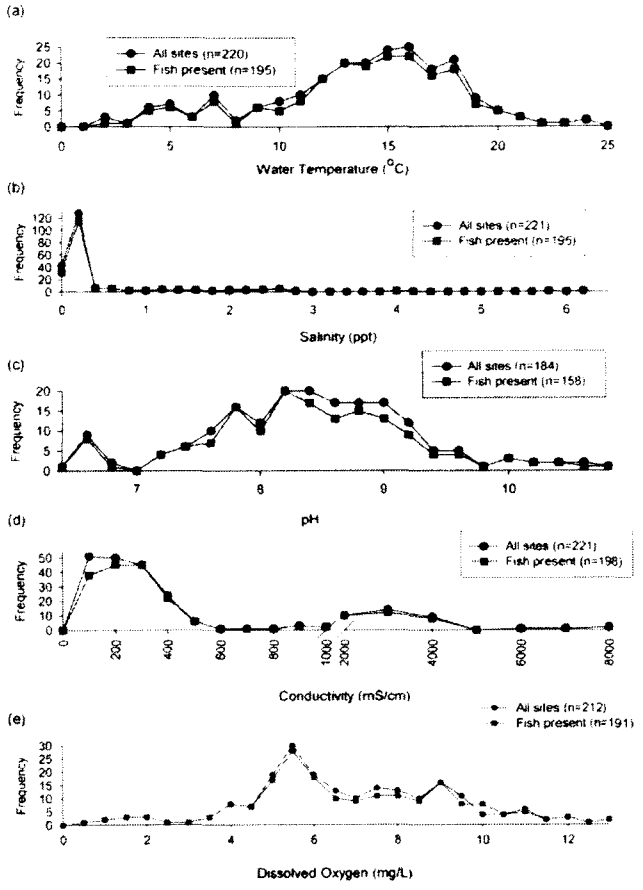

Figure 2.3. Fish species were found in a wide range of water qualities. a) Frequency of sampling of sites of different water temperatures (°C) and frequency of any fish present in those sites. b) Frequency of sampling of sites of different salinities (ppt) and frequency of any fish present in those sites. c) Frequency of sampling of sites of different pH values and frequency of any fish present in those sites. d) Frequency of sampling of sites of different conductivities (μS/cm) and frequency of any fish present in those sites. e) Frequency of sampling of sites of different dissolved oxygen values (mg/L) and frequency of any fish present in those sites.

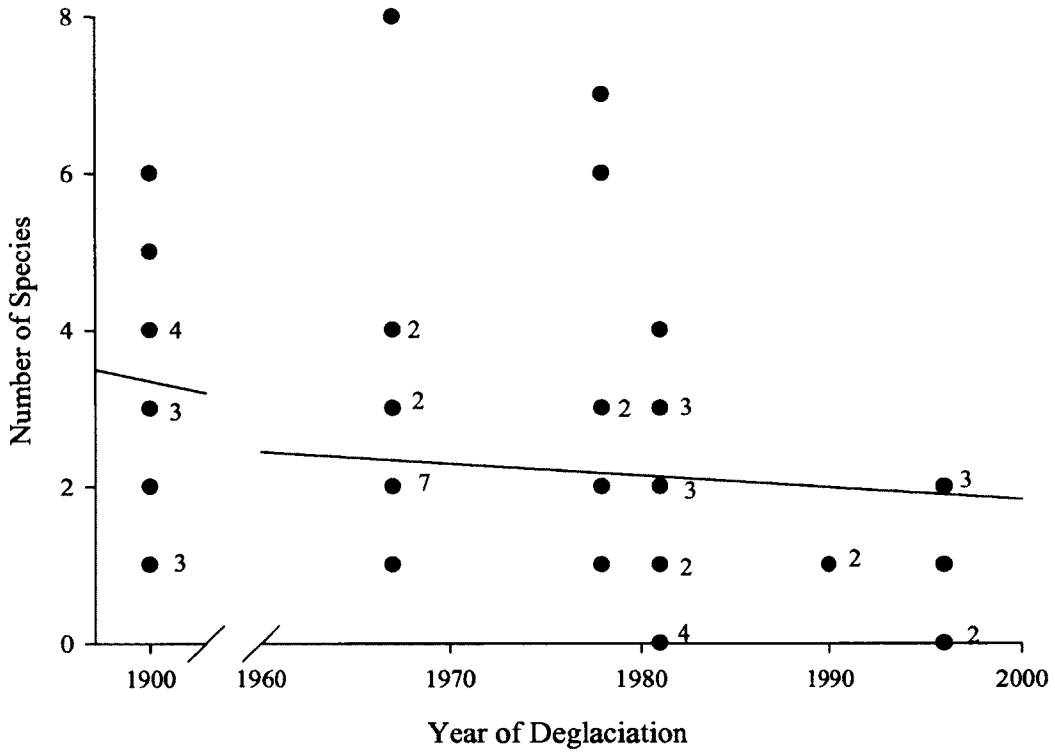

Figure 2.4. The number of fish species found in lakes and streams of different ages. Numbers next to data points indicate multiple sites with the same number of fish species. A regression line is fitted to the data, indicating a significant trend towards fewer species found in younger sites (Spearman's rho = -.460, n = 53, p = .001).

Chapter 3 Body shape variation between sympatric populations of anadromous and resident freshwater threespine stickleback in the Bering Glacier region, Alaska[1]

3.1 Abstract

Ten species pairs of anadromous and resident freshwater threespine stickleback (Gasterosteus aculeatus species complex) were discovered in six lakes and three streams in the Bering Glacier region, Alaska. One large lake (Vitus) contained two spatially separated species pairs. Resident freshwater stickleback exhibited morphological differences from anadromous stickleback based on body shape as well as meristic and linear morphometric measurements at nine sites. The body shapes of anadromous and resident freshwater members of species pairs did not covary with each other, contrary to the expectation had they recently diverged or if hybridization were common. The degree of divergence in body shape between members of a species pair was not correlated with age of the site, which also suggests a non-recent divergence. Pronounced morphological divergence, the lack of co-variation in body shape, and the lack of correlation between divergence and site age are all consistent with species pair formation by independent colonization from pre-existing populations of anadromous and resident freshwater stickleback. Sexual dimorphism in body shape is pronounced in both anadromous and

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[1] Prepared in the format for the Biological Journal of the Linnean Society. Weigner HL, FA von Hipel. (in prep).

resident freshwater populations, though the lack of covariation in body shape across the sexes suggests that different factors drive body shape evolution in each sex.

3.2 Introduction

Seven types of species pairs of threespine stickleback (Gasterosteus aculeatus species complex) with sympatric or parapatric ranges are known to naturally occur (McKinnon & Rundle, 2002; Ólafsdóttir, Ritchie, & Snorrason, 2006). Of these, only the anadromous-resident freshwater species pair is located over a wide geographic area, having originated independently and repeatedly throughout the stickleback's range (McPhail, 1994). While anadromous stickleback exhibit the ancestral oceanic phenotype, including a complete row of lateral plates, and display little morphological variation, the resident freshwater stickleback exhibit a wide range of derived phenotypes, with the low-plated condition being the most common (Bell, 1977; Bell & Foster, 1994; Walker & Bell, 2000; Spoljaric & Reimchen, 2007).

We recently discovered eight sympatric pairs of anadromous and resident freshwater threespine stickleback species in the Bering Glacier region, Alaska (von Hippel & Weigner, 2004). Since the beginning of the recession of Bering Glacier from its Holocene maximum around 1900, the region has been marked by ecological instability through periods of glacial surging events that culminate in massive outburst floods from subglacial conduits, as well as outburst floods caused by the breeching of ice dams (Fleisher, Cadwell, & Muller, 1998; Jaeger & Nittrouer, 1999). The Bering Glacier is the world's largest temperate surging glacier and has surged approximately six times since

1900 (Meier & Post, 1969; Molnia & Post, 1995). As the glacier surges, it overrides features and releases massive amounts of water and sediment (Jaeger & Nittrouer, 1999). During the most recent surge (1993-1995), the glacier advanced as rapidly as 100 meters per day (Molnia et al., 1994).

These dynamics have created a complex of freshwater habitats in which stickleback species pairs are relatively common (11% of freshwater sites sampled; Weigner & von Hippel, 2010). These pairs reside in pristine lakes and streams with widely varying ecologies (Weigner & von Hippel, 2010), which contribute to morphological diversification between populations, such as in defensive armor and trophic traits (von Hippel & Weigner, 2004).

The majority of the Bering Glacier species pairs exist in sites that were covered by ice in 1900 or later (von Hippel & Weigner, 2004). Due to the glacial surge of 1993 – 1995 and the Berg Lake outburst flood of May 1994, the Vitus Lake species pairs and the Bering River lake site species pair may have formed after those destructive events. Given that the 1993 – 1995 glacial surge into Vitus Lake destroyed the salmon runs up the Seal River (Payne et al., 1997), the possibility exists that the anadromous threespine stickleback runs were destroyed as well. This would impact species pair locations that are accessed through Vitus Lake (i.e. – Creek 1, Creek 2, and Unnamed Lake) as well as the two locations within Vitus Lake. Yet all ten species pairs appear to be breeding in sympatry with little evidence of hybridization, with the exception of Vitus Lake, in which phenotypic hybrids for lateral plate morphotype may be relatively common (von Hippel

& Weigner, 2004). Therefore, the Bering Glacier species pairs provide an exceptional opportunity to study the initial stages of speciation in nature, including the genetic, ecological, morphological, and behavioral processes involved.

This study expands upon our earlier work by using geometric morphometrics to characterize the degree of differentiation in body shape between members of the eight pairs reported by von Hippel and Weigner (2004), as well as in one more recently discovered pair. In von Hippel and Weigner (2004), we hypothesized three pathways for the origin of these species pairs: sympatric speciation, double invasion by anadromous ancestors, or independent colonization from existing resident freshwater and anadromous populations. In this study, we examine whether shape differences between anadromous and resident freshwater stickleback are consistent with any of the origin hypotheses. This study also reports on another recently discovered species pair (Tsivat Lake) for which the sample size was insufficient (n = 3 for anadromous stickleback) for shape analysis, due to number of variables in the analyses performed it is unlikely that a sample size of 3 would produce reliable results.

3.3 Methods

3.3.1 *Field Sites*

All fish were trapped in 0.32cm mesh unbaited minnow traps, semi-oval traps, seine nets and dip nets during the summers of 2003 – 2006 in the Bering Glacier region (Figure 3.1, Table 3.1). Ten sites contained threespine stickleback species pairs. In

addition, three sites that contained only resident freshwater threespine stickleback were used for comparative purposes.

3.3.2 *Morphological Variables*

Fish were killed using an overdose of MS-222 fish anesthetic and transferred to 10% buffered formalin. Fish were rinsed and bleached with 3% hydrogen peroxide and 0.5% KOH for one to two hours to remove pigmentation. They were then stained with 0.5% Alizarin Red S mixed with 1% KOH for 48 hours to aid in the visualization of bony features, and destained with 1% KOH for 12 hours to remove unwanted stain absorbed by non-bone tissues.

Morphological analyses were completed using approximately 30 males and 30 females from each anadromous and resident freshwater population, for a total of approximately 120 fish per site. The fish were randomly selected from each collection. When fewer than 30 fish of a particular type and sex were available from a site, then all available fish were used. Vitus Lake 2 had the smallest sample size of anadromous fish used in analyses (n = 19). Fish used for analyses were greater than 30mm standard length (SL: anterior tip of premaxilla to posterior border of hypural plate) because lateral plate formation is complete in fish of that size (Bell, 1981).

The left side of each fish was photographed using a Canon EOS 20D digital SLR camera. Fifteen landmarks (Figure 3.2; described by Walker, 1997) were digitized onto the image of each fish using tpsDIG2 version 2.05 (Rohlf, 2006). Landmarks were chosen for their ability to describe overall body shape (Leinonen et al., 2006).

Nonshape variation (i.e. translation, rotation, and scale) was removed using the generalized Procustes procedure (Rohlf & Slice, 1990). Partial warp and relative warp scores were calculated using the software tpsRelw (version 1.42; Rohlf, 2005a). Partial warp scores are used in multivariate analysis to describe the differences between the reference configuration and the superimposed specimens (Cavalcanti, Leandro, & Lopes, 1999). Relative warp scores are produced by an analysis that is analogous to principle components analysis (PCA) and the scores are used to summarize complex shape data into integrated variables that represent the primary axis of variation (Cavalcanti, Leandro, & Lopes, 1999; Zelditch et al., 2004). Relative warp scores and centroid sizes (multivariate measures of size used in geometric morphometrics because they are not correlated with shape; Zelditch et al., 2004) were then imported into SPSS version 11.5.0 for statistical analysis (see below). Mean configurations of body shape were also calculated for each member of a species pair (resident freshwater vs. anadromous; males and females combined) and used to test for morphological differences between these groups.

In addition to placing the landmarks, we counted the number of gill rakers on the first right gill arch and the number of lateral plates on the left side, and scored the lateral plate morph (1 = low, 2 = partial, and 3 = complete; *sensu* Bell & Foster, 1994; Bell, 2001). We also measured the horizontal orbit diameter and the lengths of the left and right pelvic spines and the second dorsal spine. Linear morphometric traits are influenced by the size of the stickleback. Therefore, we adjusted each linear trait by SL

to minimize bias due to size differences (method described in von Hippel & Weigner, 2004).

3.3.3 *Statistical Analyses*

Body shape differences were analyzed using Hotelling's T^2 tests and a combination of centroid sizes, raw scores of aligned specimens, partial warp scores, and relative warp scores as the test variables. Included in these analyses were: 1) anadromous fish from all sites vs. resident freshwater fish from all sites, 2) anadromous vs. resident freshwater individuals of each species pair, 3) the mean consensus of each anadromous population vs. the mean consensus of each resident freshwater population, 4) anadromous males from all sites vs. anadromous females from all sites, and 5) resident freshwater males from all sites vs. resident freshwater females from all sites.

Two block partial least squares (2B-PLS) analysis examines whether the axis of variation in one group is correlated with the variation in individuals from a second group. We conducted a 2B-PLS using all anadromous and all resident freshwater fish in order to determine if body shape covaried between the two life history forms regardless of site. We conducted additional 2B-PLS analyses using anadromous and resident freshwater individuals from each species pair to test for body shape covariation between members of a pair. A third 2B-PLS was used to test for covariation in body shapes of the mean configurations of each anadromous and resident freshwater population. Finally, we performed two 2B-PLS analyses, one for anadromous fish and a second for resident

freshwater fish, to test for covariation between males and females of each form. 2B-PLS analyses were performed using tpsPLS (version 1.14; Rohlf, 2005b).

Blocks were assigned *a priori* based on visual identification as anadromous or resident freshwater, or based on anatomical identification of males and females (for identification methods, see von Hippel & Weigner, 2004). Morphological verification of stickleback into either anadromous or resident freshwater classes was determined first by the examining the number of boney lateral plates, if the fish had greater than 32 plates, it was considered anadromous. The second character examined was standard length, anadromous stickleback are longer fish generally greater than 60 mm, while resident freshwater fish are between 40 – 55 mm. Examination of these two characters along with many other traits was used to determine if the fish was anadromous or resident freshwater.

2B-PLS uses a singular value decomposition (SVD) to generate pairs of singular axes (SAs; Zelditch et al., 2004). Each pair of SAs is associated with a singular value (SV) that is a relative measure of the covariance explained (Zelditch et al., 2004). These SAs, which are not associated with a significance test, are verified by performing a permutation test (9,999 iterations). If the observed singular values are greater than or equal to that which could be produced by random permutations between blocks, then the SV is considered to be non-significant (Zelditch et al., 2004). Permutation tests were also applied to the correlation coefficient (r). In order for the covariation (dimensions)

between blocks to be significant, both the singular value (λ) and the correlation coefficient (r) had to be significant (alpha = 0.05).

Linear morphometric traits and meristic traits were treated separately from geometric morphometric traits. Differences between members of each species pair were analyzed using MANOVA; sample sizes for two of the ten species pair sites were not large enough for this analysis (Tsivat River n = 3 and Vitus Lake 2 n = 20). Due to the number of variables included in these MANOVAs Tsivat River and Vitus Lake 2 were not included.

Body shape differences due to sex were visualized using thin-plate spline deformations in the "Shapes" package of the software program R (R Development Core Team, 2008). Thin-plate splines are used to graphically display shape differences, where the relative shape of one object is represented as a series of bent grid-lines superimposed over the landmark coordinates of a group mean (Bookstein, 1991).

The difference in the means of anadromous and resident freshwater fish for each species pair for the variables centroid size, Relative Warp 1, Relative Warp 2, and Relative Warp 3 were plotted against year of deglaciation to determine if a relationship existed between the difference in means and the year the lake or stream was deglaciated. The year of deglaciation was determined from GIS layers representing the terminus of the glacier at various intervals (U.S. Bureau of Land Management, 2000), including before and after surge events. The GIS layers were built from examination of aerial and LANDSAT 7 images (resolution 30 m per pixel) to determine the glacier's terminus.

3.4 Results

An anadromous and resident freshwater threespine stickleback species pair was discovered in Tsivat Lake (Figure 3.1). While adequate numbers of resident freshwater males and females were trapped, insufficient numbers of anadromous fish (n=3) were trapped for statistical analysis. It is likely that Tsivat Lake is not frequently connected to the Gulf of Alaska.

3.4.1 *Geometric Morphometric Comparison of Forms*

The Hotelling's T^2 test between all anadromous and all resident freshwater fish combined was significant ($F_{27,1,379} = 4.45$, $p<0.001$), demonstrating that the two forms are divergent in body shape. The results of the 2B-PLS analysis showed no statistically significant correlation in body shape variation between anadromous and resident freshwater fish (Table 3.2), indicating that the two forms do not vary in body shape along the same trajectory.

The Hotelling's T^2 tests between anadromous and resident freshwater members of each species pair were all significant (Table 3.3), demonstrating that the divergence between the forms occurs in all sites. The 2B-PLS analysis between anadromous and resident freshwater members of each species pair also showed no significant findings (Table 3.4), indicating that the two forms within a given pair do not vary in body shape along the same trajectory.

The Hotelling's T^2 test between the mean consensus of each anadromous population vs. the mean consensus of each resident freshwater population was also

significant ($F_{16,1} = 972.4$, $p = .025$), consistent with other results of divergence of body shape between the forms. There were also no significant findings in the 2B-PLS analysis between the mean anadromous consensus and mean resident freshwater consensus (Table 3.5).

The plot of the first two relative warps of the consensus body shape of all anadromous vs. all resident freshwater members of all species pairs indicates substantial overlap, with both forms showing large-scale variation (Figure 3.3).

3.4.2 *Linear Morphometric & Meristic Comparison of Forms*

A separate MANOVA for each species pair site revealed that anadromous and resident freshwater forms differed significantly in linear morphometric and meristic traits (Table 3.6). All seven scored linear morphometric and meristic traits contributed significantly to the differentiation of all species pairs (Table 3.6).

3.4.3 *Year of deglaciation*

To test whether body shape variation between the ancestral anadromous form and the derived resident freshwater form increases with the age of a species pair site, we examined the magnitude of the difference in the means of the centroid size and the first three relative warps against the relative age of the species pair. Relative species pair age was estimated from the year of deglaciation based on the assumption that older freshwater sites have species pairs that formed earlier. None of these comparisons between year of deglaciation and geometric morphometric values compared between

members of a species pair are significant (Figures 3.6 & 3.7; spearman rank correlations, $p > 0.05$ in all cases).

3.4.4 *Sexual Dimorphism*

The Hotelling's T^2 test between anadromous males and females showed significant differences ($F_{27,368} = 33.2$, $p < 0.001$), indicating that body shape differs between the sexes. The 2B-PLS analysis between anadromous males and females resulted in no statistically significant findings (Table 3.7), indicating that males and females do not vary in body shape along the same trajectory.

The Hotelling's T^2 test between resident freshwater males and females also showed significant differences ($F_{27,780} = 32.5$, $p < 0.001$). The 2B-PLS analysis between resident freshwater males and females also resulted in no statistically significant findings (Table 3.8).

Deformation grids of thin-plate splines were analyzed for the Tsiu River species pair because it does not contain fish of an intermediate phenotype and therefore was considered to be the most likely to be reproductively isolated (von Hippel & Weigner, 2004). The deformation grids demonstrate body shape differentiation between males and females of both anadromous and resident freshwater fish due to abdominal elongation in females, as expected for gravid fish (Figure 3.4).

3.5 Discussion

Anadromous and resident freshwater members of species pairs in the Bering Glacier region demonstrate substantial differentiation based on various linear

morphometric and meristic traits (von Hippel & Weigner, 2004), an effect that was replicated in this study (Table 3.6). Body shape also varies markedly between the two forms (Table 3.2), confirming our previous results of morphological differentiation between members of a pair (von Hippel & Weigner, 2004). The body shape of individuals of one type (anadromous or resident freshwater) within a pair did not statistically influence the body shape of individuals of the other type (Table 3.4). Similarly, the consensus body shape of all anadromous fish across all populations did not statistically influence the consensus body shape of all resident fish, or vice versa (Table 3.5).

The lack of an association in body shape between members of a species pair suggests that divergence between them was not recent (i.e. since sites formed in this newly deglaciated region). This is important because most of our study sites formed due to glacial melt after 1900 and some sites formed much more recently than that (von Hippel & Weigner, 2004). If resident freshwater fish evolved from anadromous fish recently following site formation, then we would expect significant association indicating that the ancestral anadromous form was influencing the body shape of the derived resident freshwater form. Furthermore, the lack of correlation between divergence in geometric morphometric variables and year of deglaciation (Figures 3.6 & 3.7) also suggests non-recent divergence. Therefore, these body shape results suggest that the species pairs did not evolve by sympatric speciation or by double invasion of anadromous fish following recent site formation; rather, they likely formed due to independent

colonization by pre-existing anadromous and resident freshwater populations into newly created freshwater sites (von Hippel & Weigner, 2004).

The lack of a statistical association in body shape between anadromous and resident freshwater stickleback, either within a species pair or across species pairs (Tables 3.2, 3.4, & 3.5), also suggests that hybridization between members of a pair is not common. This interpretation is consistent with other morphological results, such as the rarity of partially-plated fish in most species pair sites (von Hippel & Weigner, 2004). Therefore, it appears that anadromous and resident freshwater stickleback in species pair sites are independent populations with limited or no gene flow, i.e. separate biological species. The genetic architecture underlying their morphological differentiation remains to be explored.

Anadromous and oceanic populations throughout their range exhibit low levels of morphological diversity, while resident freshwater populations are remarkable in their divergence of forms (Walker & Bell, 2000; McKinnon & Rundle, 2002). We found, however, that both anadromous and resident freshwater members of species pairs show substantial variation in body shape (Figure 3.3), though anadromous fish differ less in linear morphometric and meristic traits (Figure 3.5).

Fish vary in body shape due to many factors. Life history traits play an important role in these differences. Because anadromous fish spend most of their lives in the ocean, they must be adapted for the oceanic environment, including possessing robust armor for predator defense and a body shape adapted for long-distance swimming. In contrast,

resident freshwater fish evolve armor and body shape related to factors such as the local predator regime, feeding ecology, and abiotic conditions, which vary widely between different lakes and streams, particularly in the dynamic Bering Glacier forelands.

Trophic traits play a role in morphological differences among individuals in a species pair. Individuals from an anadromous population are best suited for life in the littoral zone and as such they have more gill rakers to filter food and larger orbits to aid in the capture of prey. In the Bering Glacier region, resident freshwater stickleback have fewer gill rakers and smaller orbits, consistent with a benthic feeding ecology (Table 3.6).

Our results indicate the presence of sexual dimorphism in body shape as well as linear morphometric traits for both anadromous and resident freshwater stickleback (Figure 3.4; Table 3.6). The body shapes of male and female stickleback do not covary (Tables 3.7 & 3.8), indicating that different factors drive body shape evolution in each sex. Possibilities include sexual selection and sex-specific foraging patterns. Thin-plate spline deformations in the Tsiu River site indicate that sexual dimorphism in body shape relates to abdomen distention from gravidity. However, sexual dimorphism in linear morphometric traits (Table 3.6) implies that additional factors merit attention.

In sum, a combination of results – pronounced morphological divergence, lack of co-variation in body shape, and lack of correlation between divergence and site age – all point to an origin of species pairs by independent colonization from pre-existing populations of anadromous and resident freshwater stickleback. Additionally, the rarity of intermediate morphologies in species pair sites (von Hippel & Weigner, 2004) and the

lack of a statistical association in body shape between members of a pair suggest that hybridization is uncommon; the anadromous and resident freshwater members of a species pair appear to be separate biological species following their own evolutionary trajectories.

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Table 3.1. Location of populations used in analyses with GPS coordinates in decimal degrees in datum WGS 84. Differences in coordinate precision are due to the number of times the water body was sampled. All sites included species pairs except for Unnamed Pond 1 and 2 and East Tsivat Lake.

Water Body Name	Latitude (North)	Longitude (West)
Bering River lake site	60.36	143.973
Creek 1	60.103	143.36
Creek 2	60.09	143.35
Midtimber Lake	60.07	143.33
Tashalich Lake	60.039	143.63
Tsiu River	60.08	143.05
Tsivat River	60.06559	143.24696
Unnamed Lake	60.102	143.55
Vitus Lake 1	60.12186	143.28304
Vitus Lake 2	60.17406	143.26997
Unnamed Pond 1	60.455	144.2083
Unnamed Pond 2	60.11587	143.29259
East Tsivat Lake	60.13512	142.97578

Table 3.2. Results of the permutation tests for comparison of body shape of anadromous and resident freshwater stickleback from the Bering Glacier region, Alaska. Reported results are for the first five dimensions of the 2B-PLS analysis. The i^{th} singular value is λ and r_i is the correlation for the pair of vectors comprising the i^{th} dimension. Probabilities (p) are based on observed values plus 9,999 permutations of the association between anadromous and resident freshwater morphology.

Dimension	λ_i	p	r_i	p
1	0.570	0.176	0.222	0.013
2	0.190	0.635	0.131	0.822
3	0.106	0.558	0.145	0.671
4	0.051	0.792	0.188	0.102
5	0.026	0.919	0.132	0.671

Table 3.3. Results of Hotelling's T^2 analysis for comparison of differences in centroid size, relative warp scores, and partial warp scores between anadromous and resident freshwater members of nine species pairs in the Bering Glacier region. P-values are reported after the Bonferroni correction.

Species Pair	F	df	p
Bering River lake site	20.2	27,34	<0.001
Creek 1	9.4	27,82	<0.001
Creek 2	48.6	27,70	<0.001
Midtimber Lake	13.2	27,22	<0.001
Tashalich Lake	52.9	27,76	<0.001
Tsiu River	148.2	27,94	<0.001
Unnamed Lake	46.1	27,56	<0.001
Vitus Lake 1	21.0	27,46	<0.001
Vitus Lake 2	12.6	27,12	<0.001

Table 3.4. Results of the permutation tests for comparison of body shape of anadromous and resident freshwater stickleback in all species pairs from the Bering Glacier region, Alaska. Reported results are for the first five dimensions of the 2B-PLS analysis. The i^{th} singular value λ_i and r_i is the correlation for the pair of vectors comprising the i^{th} dimension. Probabilities (p) are based on observed values plus 9,999 permutations of the association between anadromous and resident freshwater morphology.

Population	λ_i	p	r_i	p
Bering River lake site				
Dimension 1	0.622	0.271	0.565	0.216
Dimension 2	0.223	0.433	0.555	0.184
Dimension 3	0.070	0.800	0.533	0.265
Dimension 4	0.042	0.648	0.656	0.027
Dimension 5	0.016	0.919	0.507	0.466
Creek 1				
Dimension 1	0.489	0.678	0.358	0.721
Dimension 2	0.238	0.349	0.351	0.638
Dimension 3	0.132	0.143	0.404	0.487
Dimension 4	0.044	0.620	0.518	0.097
Dimension 5	0.028	0.581	0.445	0.394
Creek 2				
Dimension 1	0.444	0.760	0.440	0.309
Dimension 2	0.259	0.217	0.338	0.871
Dimension 3	0.110	0.401	0.448	0.362
Dimension 4	0.047	0.696	0.453	0.352
Dimension 5	0.040	0.314	0.416	0.559
Midtimber Lake				
Dimension 1	0.590	0.349	0.650	0.101
Dimension 2	0.186	0.540	0.552	0.598

Table 3.4 Continued

Population	λ_i	p	r_i	p
Dimension 3	0.085	0.620	0.666	0.279
Dimension 4	0.054	0.526	0.695	0.133
Dimension 5	0.030	0.640	0.572	0.618
Tashalich Lake				
Dimension 1	0.677	0.058	0.462	0.243
Dimension 2	0.116	0.958	0.395	0.606
Dimension 3	0.066	0.904	0.371	0.764
Dimension 4	0.045	0.772	0.607	0.006
Dimension 5	0.031	0.663	0.539	0.067
Tsiu River				
Dimension 1	0.462	0.671	0.606	<0.001
Dimension 2	0.272	0.186	0.279	0.963
Dimension 3	0.104	0.504	0.492	0.036
Dimension 4	0.083	0.135	0.340	0.700
Dimension 5	0.032	0.560	0.458	0.083
Tsivat River				
Dimension 1	0.671	0.677	0.982	0.667
Unnamed Lake				
Dimension 1	0.466	0.639	0.487	0.362
Dimension 2	0.207	0.473	0.376	0.926
Dimension 3	0.128	0.263	0.526	0.265
Dimension 4	0.083	0.170	0.464	0.542
Dimension 5	0.030	0.733	0.528	0.178
Vitus Lake 1				
Dimension 1	0.610	0.282	0.519	0.175
Dimension 2	0.169	0.671	0.577	0.088
Dimension 3	0.081	0.648	0.526	0.351
Dimension 4	0.056	0.434	0.501	0.550

Table 3.4 Continued

Population	λ_i	p	r_i	p
Dimension 5	0.031	0.574	0.460	0.726
Vitus Lake 2				
Dimension 1	0.480	0.758	0.486	0.905
Dimension 2	0.354	0.019	0.701	0.089
Dimension 3	0.052	0.899	0.653	0.260
Dimension 4	0.040	0.643	0.570	0.661
Dimension 5	0.031	0.332	0.570	0.677

Table 3.5. Results of permutation tests for comparisons of body shape between mean anadromous and mean resident freshwater members of species pairs from the Bering Glacier region. Reported results are for the first five dimensions of the 2B-PLS analysis. The i^{th} singular value λ_i and r_i is the correlation for the pair of vectors comprising the i^{th} dimension. Probabilities (p) are based on observed values plus 9,999 random permutations.

Dimension	λ_i	p	r_i	p
1	0.751	0.222	0.859	0.100
2	0.160	0.736	0.707	0.480
3	0.048	0.777	0.654	0.578
4	0.035	0.265	0.718	0.293
5	0.004	0.911	0.702	0.404

Table 3.6. MANOVAs from eight of the Bering Glacier region species pairs based on seven linear morphological and meristic traits. The Tsivat River and Vitus Lake 2 pairs were not analyzed due to low sample sizes. Reported here are the F's from a MANOVA based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits (see von Hippel and Weigner 2004). Type (anadromous or resident freshwater) and sex were fixed factors. Sex was reported when significant. The interaction of type by sex was not significant for any species pairs. P-values are reported after the Bonferroni correction.

Species Pair	F	df	p	Partial η^2
Type				
Bering River lake site	50.4	7,29	<0.001	.924
Creek 1	184.9	7,96	<0.001	.931
Creek 2	336.7	7,80	n.s.	.967
Midtimber Lake	50.8	7,38	<0.001	.903
Tashalich Lake	5,798.7	6,92	<0.001	.997
Tsiu River	5,317.3	6,104	<0.001	.997
Unnamed Lake	317.9	7,72	<0.001	.969
Vitus Lake 1	205.1	7,58	<0.001	.961
Sex				
Creek 1	4.0	7,96	0.01	.227
Tashalich Lake	7.6	6,92	<0.001	.332
Tsiu River	3.2	6,104	0.05	.156
Unnamed Lake	4.4	7,72	0.01	.299

Table 3.7. Results of a permutation test for a comparison between anadromous males and anadromous females. Reported results are for the first five dimensions of the 2B-PLS analysis. The i^{th} singular value λ_i and r_i is the correlation for the pair of vectors comprising the i^{th} dimension. Probabilities (p) are based on observed values plus 9,999 random permutations.

Dimension	λ_i	p	r_i	p
1	0.420	0.740	0.248	0.220
2	0.270	0.103	0.246	0.420
3	0.097	0.557	0.261	0.439
4	0.077	0.227	0.304	0.124
5	0.050	0.249	0.323	0.031

Table 3.8. Results of permutation tests for a comparison of resident freshwater males and females. Reported results are for the first five dimensions of 2B-PLS analysis. The i^{th} singular value λ_i and r_i is the correlation for the pair of vectors comprising the i^{th} dimension. Probabilities (p) are based on observed values plus 9,999 random permutations.

Dimension	λ_i	p	r_i	p
1	0.495	0.248	0.177	0.540
2	0.205	0.576	0.162	0.672
3	0.105	0.702	0.150	0.741
4	0.063	0.713	0.158	0.575
5	0.043	0.604	0.112	0.983

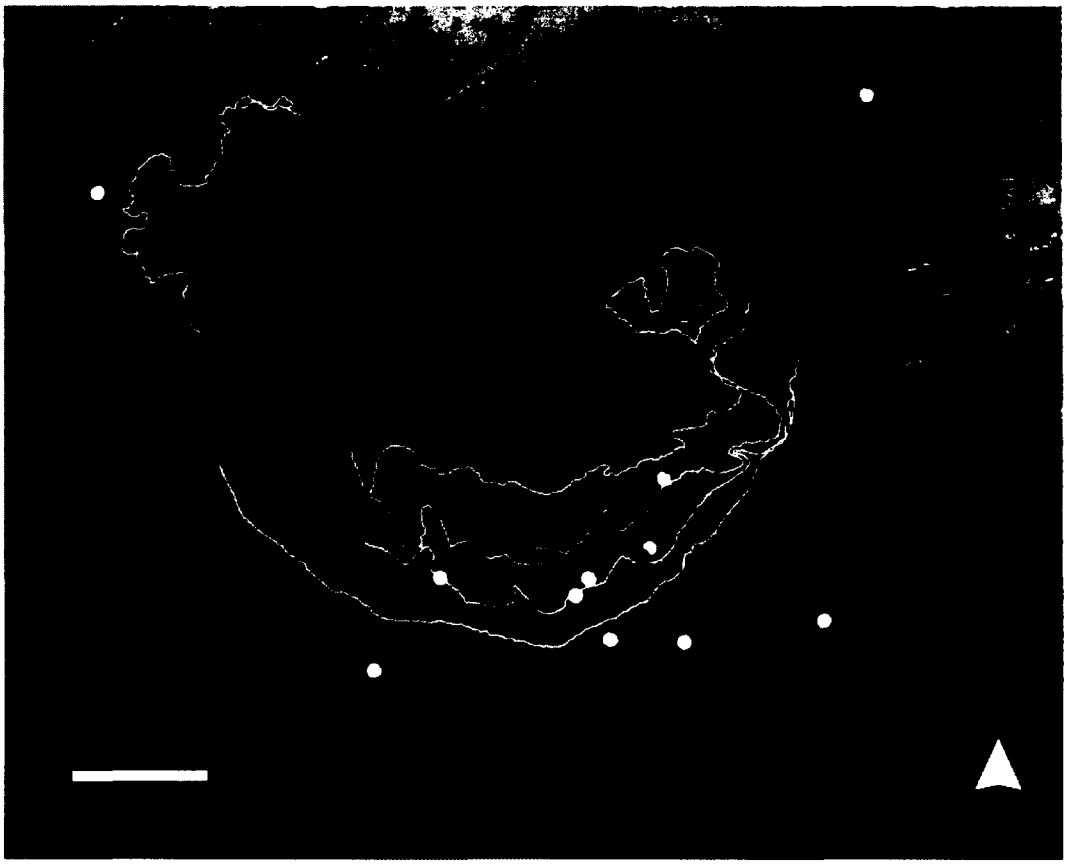
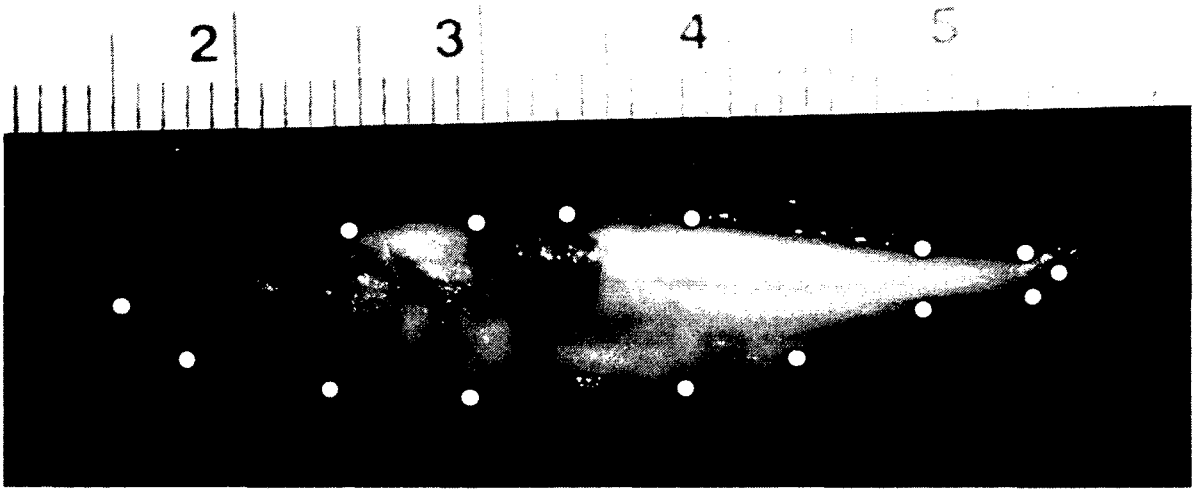


Figure 3.1. Landsat imagery of the Bering Glacier region, Alaska, showing all populations used in analyses. Yellow circles indicate species pair locations and pink circles indicate outside resident freshwater populations. White lines indicate the position of the glacier's terminus in the years 1900, 1967, 1993 and 1995. Image was captured September 10, 2001.

a)



b)

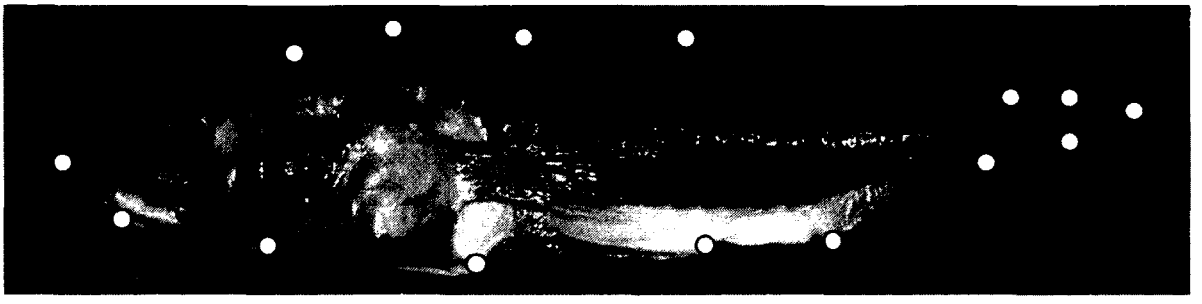


Figure 3.2. Landmark positions on threespine stickleback: a) resident freshwater, b) anadromous.

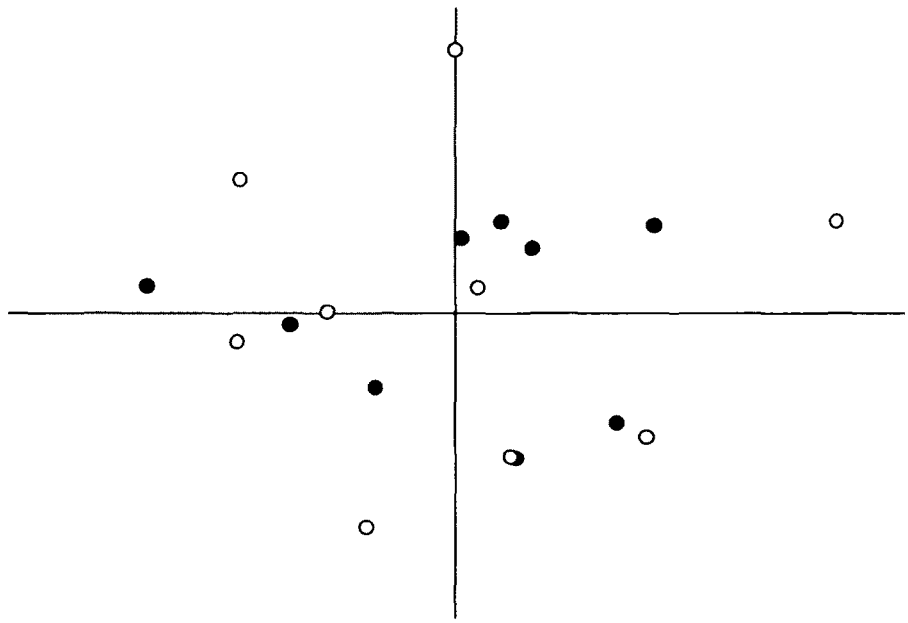
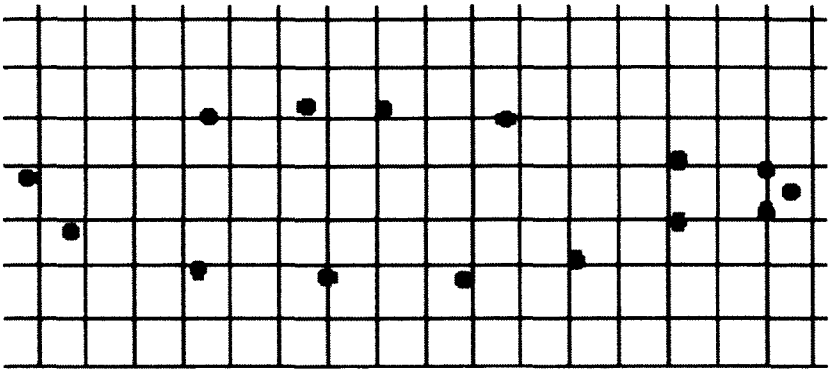


Figure 3.3. Relative warp 1 plotted against relative warp 2 for anadromous (closed circles) and resident freshwater (open circles) members of species pairs.

a)



b)

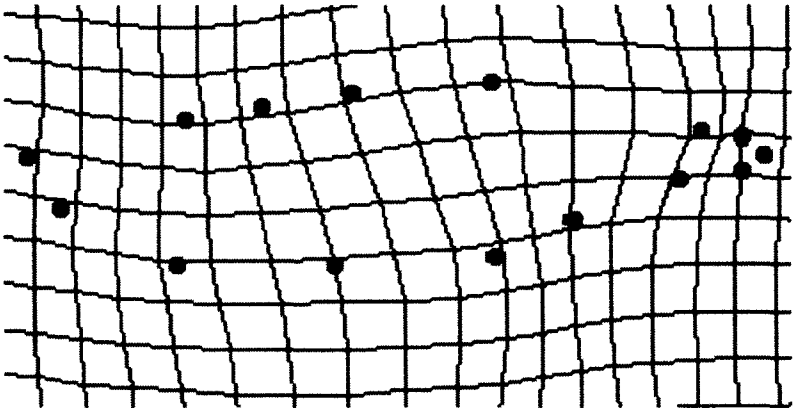
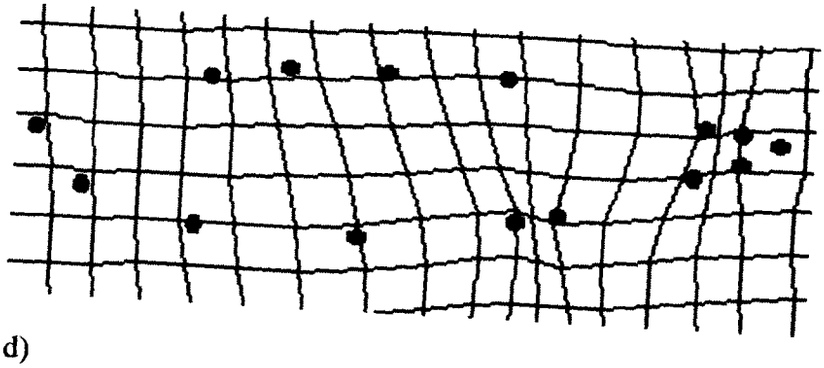
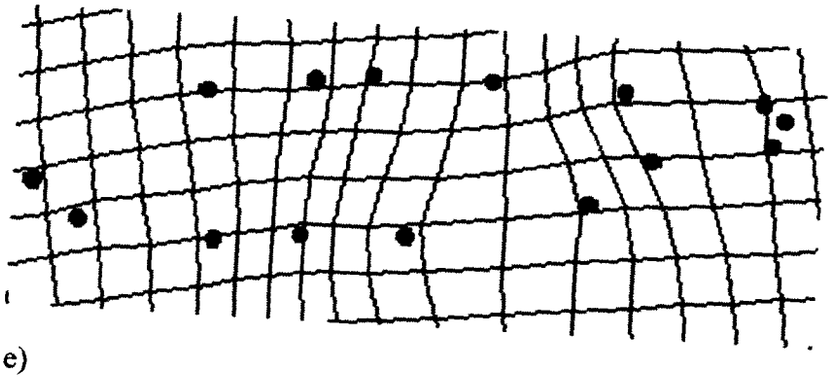


Figure 3.4. Overall deformation grid of Tsiu River threespine stickleback a) mean body shape of all stickleback, b) mean anadromous females, c) mean anadromous males, d) mean resident freshwater females, e) mean resident freshwater males.

c)



d)



e)

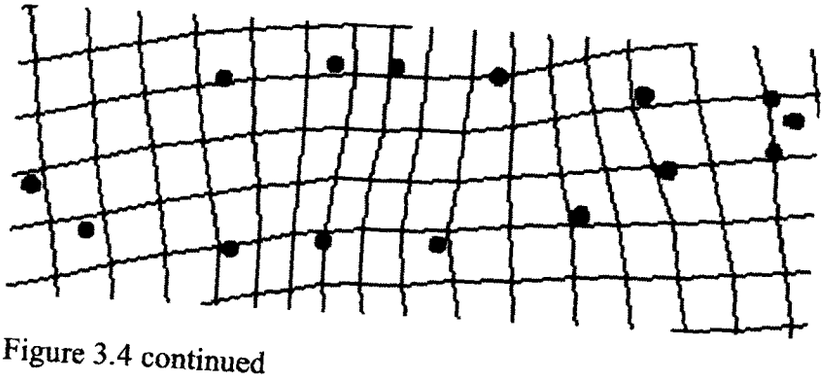


Figure 3.4 continued

a)

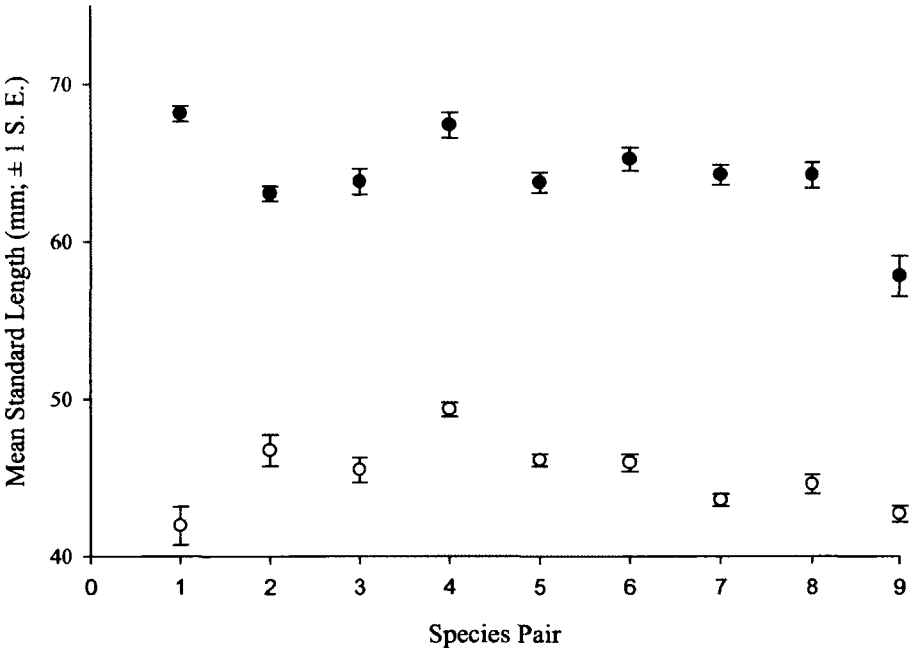


Figure 3.5. Means ± 1 SE of a) standard length, b) left side lateral plate number, and c) number of gill rakers for anadromous (solid points) and resident freshwater (open points) members of species pairs. Species pairs listed by number correspond to: 1 = Bering River Lake Site, 2 = Creek 1, 3 = Creek 2, 4 = Midtimber Lake, 5 = Tashalich Lake, 6 = Tsiu River, 7 = Unnamed Lake, 8 = Vitus Lake 1, and 9 = Vitus Lake 2. The Tsivat species pair site is not included due to small sample size of anadromous fish.

b)

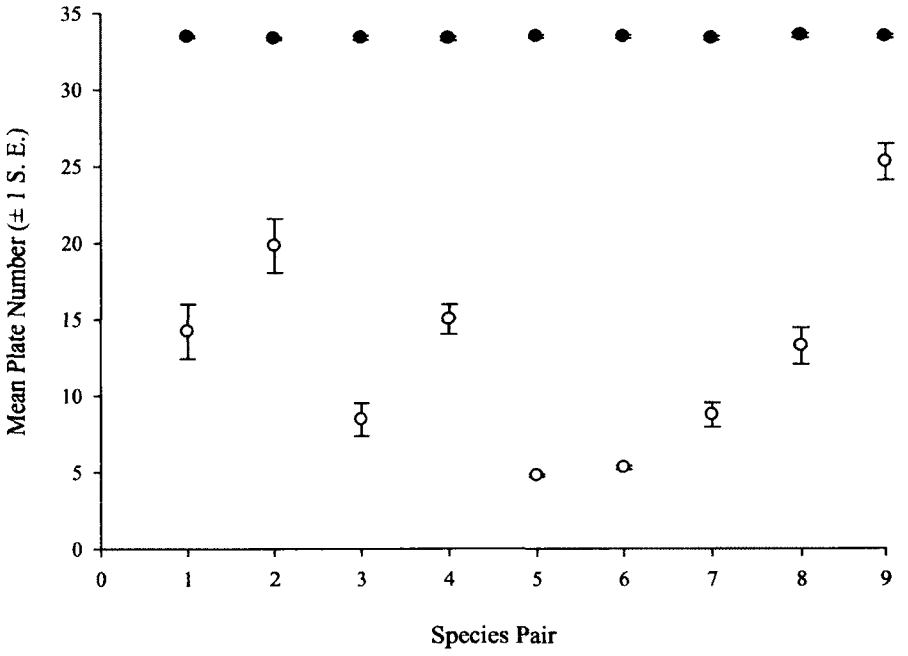


Figure 3.5 continued

c)

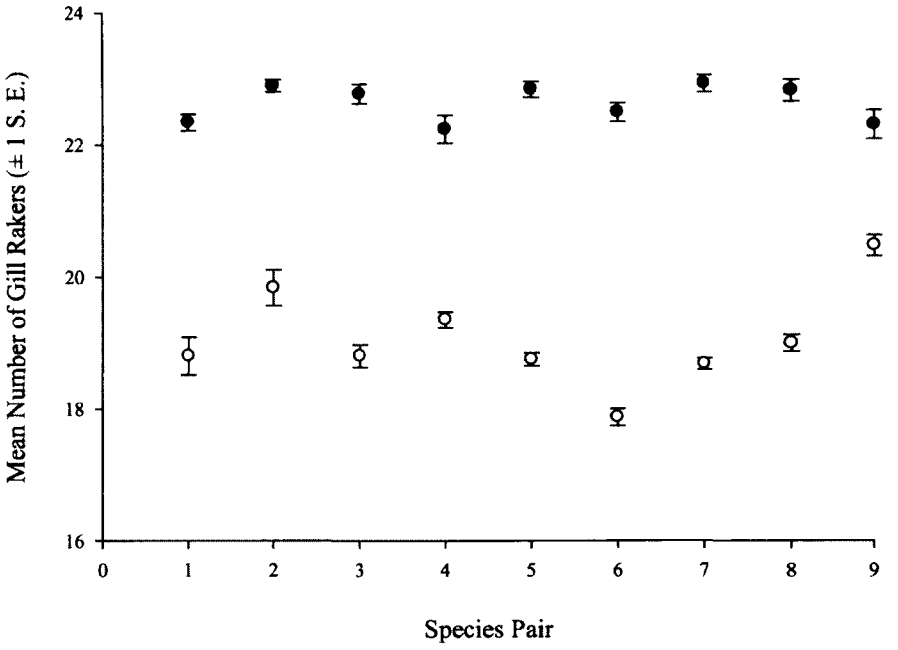


Figure 3.5 continued

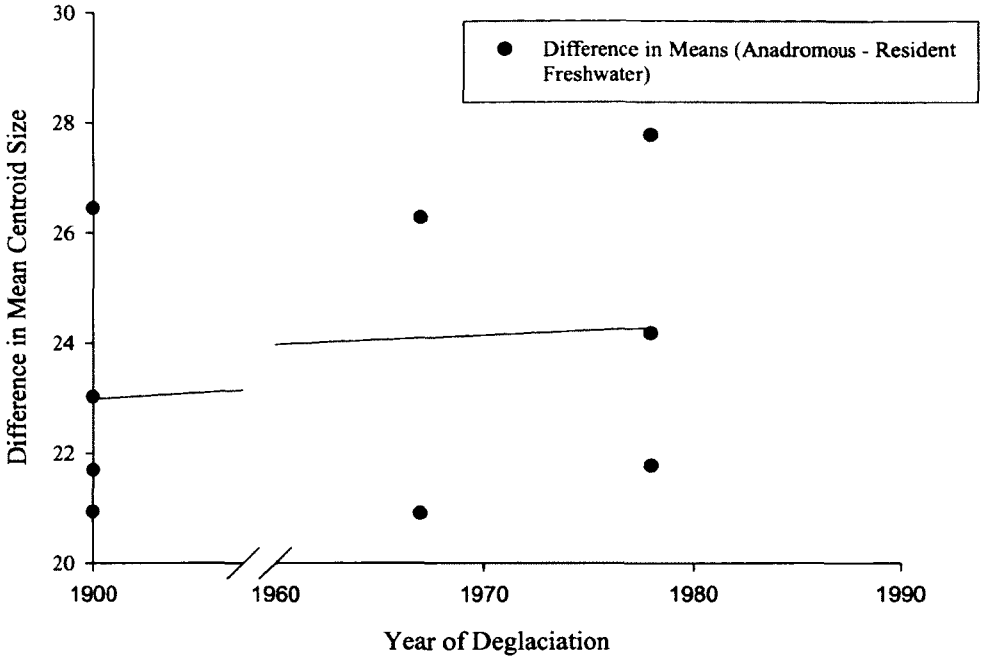


Figure 3.6. Differences in anadromous and resident freshwater threespine stickleback means for centroid size and year of deglaciation.

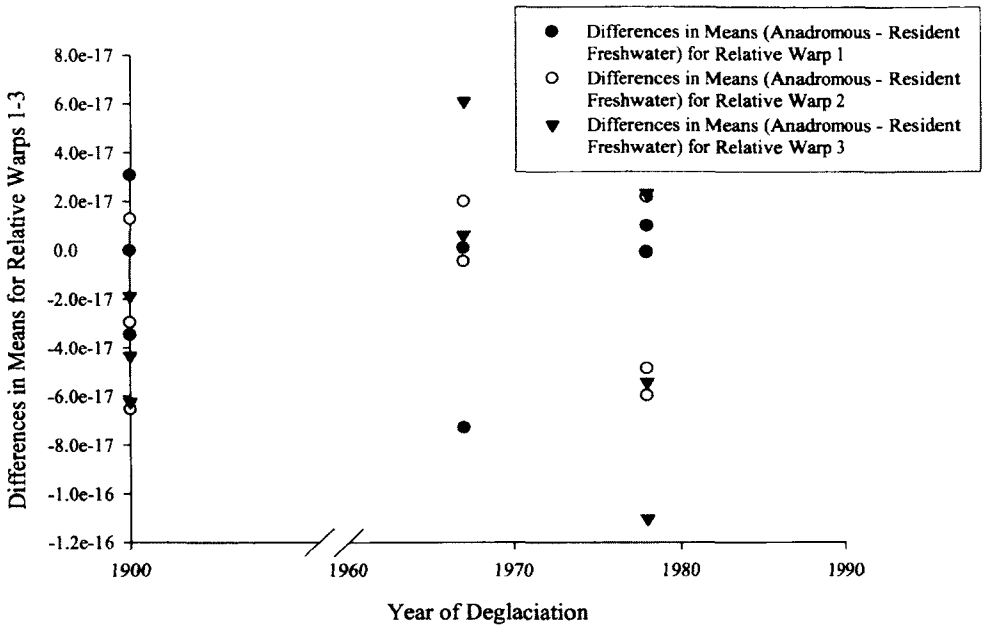


Figure 3.7. Differences in anadromous and resident freshwater threespine stickleback means for relative warps 1 – 3 and year of deglaciation.

Chapter 4 Genetic structure of anadromous and resident freshwater species pairs of threespine stickleback in the Bering Glacier region, Alaska[1]

4.1 Abstract

Ten populations of anadromous and resident freshwater threespine stickleback species pairs were discovered in the Bering Glacier region, Alaska. Three hypotheses are explored for the origin of the species pairs: sympatric diversification by anadromous stickleback, double invasion by anadromous stickleback, and independent colonization from pre-existing anadromous and resident freshwater populations. Two mitochondrial DNA (mtDNA) stickleback clades are found in these populations, the Euro-North American clade (ENAC) and the Trans-North Pacific clade (TNPC). The ENAC is the most commonly found clade in both resident freshwater and anadromous stickleback, though the TNPC is found more often in resident freshwater populations (13 – 83% of fish in resident freshwater populations vs. 0 – 8% in anadromous populations). Three resident freshwater populations were composed of more TNPC fish than ENAC fish; each of these was a water body outside of the Little Ice Age glacial maximum, suggesting that the TNPC may have colonized the region first. The differences in clade frequencies between resident freshwater and anadromous populations support the independent

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[1] Prepared in the format for the Biological Journal of the Linnean Society. Weigner HL, FA von Hippel, N Nishimure, WA Cresko. (in prep).

colonization hypothesis. A portion of the mtDNA gene NADH2 was sequenced on a subset of these populations. Based on the phylogenetic sequence analysis, limited genetic structure exists in these populations. While the sequence analysis supports the independent colonization hypothesis for the Tashalich Lake species pair, it does not support or refute the hypothesis for other pairs.

4.2 Introduction

The threespine stickleback (*Gasterosteus aculeatus* species complex) is a model organism in studies of speciation in part because of the existence of reproductively isolated species pairs, extant ancestral and derived phenotypes, and compelling evidence of rapid and parallel speciation (Bell & Foster, 1994). Seven varieties of stickleback species pairs with sympatric or parapatric ranges are known to occur (McKinnon & Rundle, 2002; Ólafsdóttir, Ritchie, & Snorrason, 2006). Of these, only the anadromous-resident freshwater pair is distributed over a wide geographic area (McPhail, 1994), having repeatedly originated throughout the stickleback's Holarctic range. Anadromous stickleback exhibit the ancestral oceanic phenotype with little morphological variation, whereas the resident freshwater stickleback exhibit a wide range of derived phenotypes (Bell, 1977; Bell & Foster, 1994; Walker & Bell, 2000; Spoljaric & Reimchen, 2007).

The life history of threespine stickleback involves colonization of newly formed freshwater habitat by anadromous stickleback (McKinnon & Rundle, 2002). After colonization the stickleback populations experience rapid changes in life history, morphology, behavior, and physiology as they adapt to the freshwater environment

(McKinnon & Rundle, 2002). Most morphological and genetic diversity exists in the resident freshwater threespine stickleback; however, diversity within some marine forms exist (McKinnon & Rundle, 2002). Speciation in stickleback is most likely driven by divergergent selection (McKinnon & Rundle, 2002).

In our previous work, we described species pairs of anadromous and resident freshwater stickleback from diverse habitats in the Bering Glacier region, Alaska (von Hippel & Weigner, 2004; Weigner & von Hippel, 2010). We proposed three possible origins for the Bering Glacier species pairs: sympatric speciation from an anadromous ancestor, double invasion by anadromous ancestors, or independent colonization of newly created lakes and streams by resident freshwater and anadromous fish.

For sympatric speciation to occur, some offspring of anadromous fish must fail to return to the sea, and mate assortatively, while anadromy is retained by other stickleback from the same population. This might occur through major selection differentials acting on opposite sides of an ecotone (McPhail, 1994), but such disruptive selection seems unlikely in the Bering Glacier region where the locations of ecotones constantly shift due to abiotic disturbance (von Hippel & Weigner, 2004). Double invasion could occur if a geologic or hydrologic event isolated anadromous fish from the ocean, leading to directional selection to a freshwater phenotype. At a later time, when the water body regains contact with the ocean, it could be colonized by anadromous stickleback again, thus forming a species pair of anadromous and resident freshwater stickleback (von Hippel & Weigner, 2004). Independent colonization could have occurred when the

Bering Glacier began to recede from its Neoglacial maximum, around 1900, or subsequent to that. Colonization of newly formed water bodies by resident freshwater stickleback from pre-existing lakes and streams may have occurred before or after the independent colonization of these water bodies by anadromous stickleback from the Gulf of Alaska (von Hippel & Weigner, 2004).

In this study, we test these hypotheses for the origin of the Bering Glacier species pairs, and we analyze the colonization history of the two major mitochondrial clades of the cytochrome b gene in the region. We also compare the clade frequencies with the approximate year of deglaciation for each water body. We present sequence data for a region of the mtDNA gene NADH 2 to determine genetic divergence between anadromous and resident freshwater members of two species pairs.

Previous work on the cytochrome b gene identified two clades in the threespine stickleback; the Trans-North Pacific Clade (TNPC; also named 'Argonaut', O'Reilly et al., 1993; 'Japanese', Ortí et al., 1994) and the Euro-North American Clade (ENAC; Ortí et al., 1994; Johnson & Taylor, 2004). These clades diverged approximately two million years before present when the Sea of Japan was isolated from the Pacific Ocean (Higuchi & Goto, 1996). The ENAC haplotype lineage is located in freshwater and marine environments throughout Europe and North America (Ortí et al., 1994; Deagle, Reimchen, & Levin, 1996; Thompson, Taylor, & McPhail, 1997; Johnson & Taylor, 2004). The TNPC haplotype lineage is located in freshwater and marine environments in the Sea of Japan and the Sea of Okhotsk (Higuchi & Goto, 1996) and occurs relatively

infrequently in the eastern Pacific; however, in some lakes and streams along western North America stickleback with the TNPC haplotype lineage reach a high frequency, with the highest found in Rouge Lake, British Columbia at 100% (Deagle et al., 1996; Thompson et al., 1997; Johnson & Talyor, 2004). In this paper we examine the frequencies of both clades for seven of the ten species pair sites, as well as three nearby resident freshwater sites.

4.3 Study Area

The Bering Glacier is the world's largest temperate surging glacier, with surges occurring approximately every 20 – 30 years (Post, 1972). Surges for the Bering Glacier occurred at approximately 1900, approximately 1920, 1938-1940, 1957-1967, and 1993-1995 (Molnia & Post, 2010). As the terminus of the glacier surges forward, it overrides the young landscape. The time periods in between surging events are marked by glacial recession.

As the glacier moves through the Bagley Icefield, it divides into the Bering and Stellar lobes. The termini of these lobes form a region of proglacial lakes (Figure 4.1; Molnia & Post, 1995; Herzfeld & Mayer, 1997) with Vitus Lake being the largest. With the exception of the Bering River lake site, all species pairs were found in the Bering Glacier foreland, which drains the Bering Lobe (Figure 4.1).

Vitus Lake did not exist prior to 1900, the Neoglacial maximum of Bering Glacier (Molnia & Post, 1995). As the glacier began to recede, Vitus Lake formed in its wake; however, due to the 1993-1995 glacial surge, which reduced the area of Vitus Lake to 50

km^2 (Molnia & Post, 2010), the Vitus Lake species pair site may have formed as recently as 1995. Presently, Vitus Lake is 23 km long with a maximum width of 8.5 km and covers an area of 160 km^2 (Molnia & Post, 2010). Tashalich Lake, was a proglacial river in 1900, and hence the Tashalich species pair is potentially older than 1900. As Vitus Lake grew in size, Tashalich River drained the lake instead of the glacier directly. Currently, approximately 600 meters separate Vitus Lake and Tashalich Lake (Molnia & Post, 2010). As of 2005, Tashalich Lake had a small, continual opening to the Gulf of Alaska (H. L. Weigner pers. obs.).

4.4 Methods

4.4.1 *Sample Collection & Processing*

Fish were trapped May – August, 2004 - 2006, using 0.32 cm unbaited mesh minnow traps, dip nets, and a seine net. Fish were killed with an overdose of MS-222 anesthetic, and stored at -80°C or preserved in 95% ethanol. Forty-eight stickleback from each anadromous and resident freshwater population were randomly selected for DNA extraction; however, if 48 stickleback were not collected from a population, then as many stickleback as available were used. Total genomic DNA was extracted from caudal fin clips using the EPICENTRE MasterPure™ DNA Purification Kit.

4.4.2 *Mitochondrial Haplotype Analysis*

The polymerase chain reaction (PCR) was used to amplify a section of the cytochrome b mitochondrial gene for the TNPC and ENAC haplotype analysis. Primers were designed specifically for threespine stickleback using Lasergene®PrimerSelect™

and selected based on score, length of the product, and percent of GC nucleotides. Primers used were L14327 5' – CGACTTGAAAAACCATCGTTG – 3' and H15150 5' – TTTGCAGGGGTGAAGTTATCAGGAT – 3' as well as L14372 5' – ATGGCAAGCCTACGAAAAACGCAC – 3' and H15100 5' – TGCTAGGGATGTAAGGGCAATTAG – 3'. DNA was amplified in a 50μL total volume reaction, containing 1.25 μL 100 mM $MgSO_4$, 0.5 μL 1M KCl, 1.0 μL 1M $(NH_4)_2SO_4$, 0.5 μL 1M Tris-HCl (pH 8.8), 0.5 μL 1M Tris-HCl (pH 8.4), 0.5 μL 10% Triton X-100, 0.25 μL DMSO, 0.05 μL 100mg/mL BSA, 0.25 μL 10 μM dNTP, 20.2 μL water, 0.25μL of a forward and reverse primer, 0.625μL *Taq*, 18.875μL nanopure water, and 5.0 μL DNA template. A touchdown from 64°C to 56°C was performed for 16 cycles. The thermocycler settings were 94°C for 1 minute, 56°C for 1 minute and 72°C for 2 minutes for 37 cycles. The bands produced were 823bp (produced by primer pair L14327 and H15150) and 728bp long (produced by primer pair L14372 and H15100). The primer pair L14327 and H15150 was selected first for use in the reactions. If the reaction failed, then the second primer pair was employed.

PCR products were digested with BstXI and NlaIII (New England Biolabs). PCR products were digested in 5.75 μL total volume reaction, containing 2.5 μL 10x enzyme buffer, 0.25 μL enzyme, and 3.0 μL nanopure water, and digested overnight. BstXI cuts the PCR product and produces two bands in the ENAC (approximately 500 and 300 bp long); however, the TNPC fish are lacking a restriction site for BstXI (Cresko, 2000). Fish that lacked the two bands from the BstXI digestion were then digested with NlaIII to

confirm their TNPC identity. NlaIII produces four fragments in fish from TNPC (approximately 40, 130, 260, and 300 bp each) and two in the ENAC. In the TNPC fish, no difference was seen in the 260 bp and 300 bp fragments on the gel and the 40 bp fragment did not show up on the gel. DNA sequencing was performed to determine the exact number of restriction sites for the NlaIII and their locations. Products of the restriction enzyme digest were separated by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized with ultraviolet light.

RFLP data were analyzed using Arlequin 3.5.1.3 (Excoffier & Lischer, 2010). Haplotype frequencies were calculated for all 17 populations of threespine stickleback from both clades (Figure 4.2). Population pairwise comparisons using haplotype frequencies were used to calculate conventional F_{ST} values (Table 4.2), *p*-values were corrected using the Bonferroni correction.

Frequency of ENAC in all 17 populations was plotted against the approximate year of deglaciation. The year of deglaciation was determined from GIS layers representing the terminus of the glacier at various intervals, including before and after surge events (U.S. Bureau of Land Management, 2000). The GIS layers were built from examination of aerial and LANDSAT 7 images (resolution 30 m per pixel) to determine the glacial terminus.

4.4.3 *NADH2 Sequence Analysis*

Two out of the ten identified species pairs in the Bering Glacier region were selected for sequencing of a portion of the NADH 2 gene based on age of the water body and sample size.

PCR was used to amplify a portion of the mtDNA NADH2 gene specified by the oligonucleotide primers Forward 5' – GTCTTCCACCACAAGTGTAAACG – 3' and Reverse 5' – CGTTACCTTCTCATCCGATCCT – 3' as well as Forward 5' - GTCTTCCACCACAAGTGTAAACG – 3' and Reverse 5' – CGTTACCTTCTCATCCGATCCT – 3', which were designed in Lasergene®PrimerSelect™ and Geneious (Drummond et al., 2011) based on score, product length, and percent of GC nucleotides. DNA was amplified in a 20 μL total volume reaction consisting of 10 μL 2x turbo (see methods above), 0.1 μL forward primer, 0.1 μL reverse primer, 0.15 μL *Taq*, 7.65 μL nanopure water, and 2.0 μL DNA template. The thermocycler settings were 94°C for 6 minutes, followed by 94°C for 1 minute, 58°C for 1 minute, 72°C for 2 minutes for 37 cycles, followed by 72°C for 2 minutes. The PCR product was purified using Zymo DNA Clean & Concentrator™ – 5. After purification, a total volume of 6 μL (5.5 μL purified PCR product and 0.5 μL of forward primer) were submitted for sequencing to the University of Oregon DNA sequencing facility.

Sequence data were edited and aligned in Geneious 5.3.6 (Drummond et al., 2011). Total alignment length was 1237 bp. GenBank sequences from a threespine

stickleback (Accession Number NC_003174), a blackspotted stickleback (*Gasterosteus wheatlandi*, Accession Number NC_011570) and a ninespine stickleback (*Pungitius pungitius*, Accession Number NC_011571) were downloaded to represent an additional group. Because all samples were not sequenced for the whole region only a 195 overlapping bp region was used in the analyses.

Phylogenetic trees were constructed in MEGA version 5 (Tamura et al., 2011). A maximum likelihood test was used to determine the model of nucleotide substitution to use in constructing the tree. A maximum likelihood tree with 1,000 bootstrap replicates was computed using the Hasegawa-Kishino-Yano model with invariant sites (Hasegawa, Kishino, & Yano, 1985). A minimum evolution method based on γ_{ST} was used to build a population level tree.

DnaSP 5.10 (Librado & Rozas, 2009) was used to calculate indices of pair-wise genetic distance and diversity. Indices of genetic distance were performed with 1,000 permutations. Arlequin 3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate analysis of molecular variance (AMOVA) and pairwise F_{ST}. AMOVAs were performed to assess the total nucleotide variance between groups, among populations within groups, and within populations. Bering Glacier sites were analyzed by anadromous vs. resident freshwater populations, and by Vitus Lake (anadromous and resident freshwater) vs. Tashalich Lake (anadromous and resident freshwater). P-values from the pairwise F_{ST} values were adjusted with the Bonferroni correction.

Nucleotide diversity (π) was plotted against the year of deglaciation to determine if greater nucleotide diversity exists in older populations.

4.5 Results

4.5.1 *Mitochondrial Haplotype Analysis*

Only three populations, the resident freshwater fish from East Tsivat Lake, Tashalich Lake, and Tsivat Lake, had a higher frequency of the TNPC than the ENAC (Figure 4.2). All three of these populations exist outside of the Little Ice Age glacial maximum, so all three are potentially older than 1900. The frequency of the TNPC is much higher in resident freshwater populations than in anadromous populations, regardless of whether it is a species pair site or not (Figure 4.2).

Pairwise F_{ST} values based on mtDNA clade haplotypes between anadromous populations were all non-significant (Table 4.2), indicating little genetic distance between anadromous populations. Some resident freshwater-resident freshwater and resident freshwater-anadromous pairwise F_{ST} values were significant (Table 4.2).

There were no clear patterns in the frequency of ENAC occurrence by the year of deglaciation (Figure 4.3). The oldest sites, which have been deglaciated before 1900, had both the highest (0.96 - 1.0) and lowest (0.17) frequency of ENAC.

4.5.2 *NADH2 Sequence Analysis*

A maximum likelihood phylogenetic tree showed two of the three sequences from the additional group sequences (ninespine and blackspotted stickleback) are the most diverged from the Bering Glacier populations (Figure 4.4). The third sequence from a

threespine stickleback clustered with Tashalich Lake anadromous fish. There was limited structuring in the tree. It should be noted that four of the five Tashalich Lake resident freshwater sequences were grouped together with four Vitus Lake resident freshwater sequences and two Tashalich Lake anadromous sequences (Figure 4.4).

The minimum evolution model phylogenetic tree based on γ_{ST} values shows Vitus Lake anadromous and resident freshwater populations as being the most closely related (Figure 4.5). Tashalich Lake anadromous and resident freshwater populations are more closely related to each other than they are to the Vitus Lake populations.

In the Bering Glacier populations, there were four unique haplotypes among the 69 sequences ($h_d = 0.349$), indicating that NADH2 is conserved across populations. Nucleotide diversity (π) was also low throughout the Bering Glacier region ($\pi = 0.008$), indicating few nucleotide differences between sequences. Tashalich Lake resident freshwater fish had the greatest values for π and h_d, while Vitus Lake anadromous fish had the lowest (Table 4.3). While nucleotide diversity (π) was not great in any population, it was greater in both resident freshwater populations than in anadromous populations.

Based on pairwise F_{ST} values derived from NADH2 sequence data, Tashalich Lake freshwater fish significantly differ from all other populations, except for the outgroup, while all other populations only significantly differ from the outgroup + Tashalich Lake (Table 4.4).

The analysis of structure between anadromous and resident freshwater populations, as well as between Vitus Lake and Tashalich Lake populations, indicates that the majority of variation (73% and 75%) occurs within populations (Tables 4.5 & 4.6). Large amounts of variance (51% and 52%) also exist among populations within groups (Tables 4.5 & 4.6). Negative variance components were generated between groups (Tables 4.5 & 4.6), which can be interpreted as a zero component (Cresko, 2000).

There was no clear relationship between nucleotide diversity and the year of deglaciation (Figure 4.6). Although the Tashalich Lake resident freshwater population had the greatest nucleotide diversity and Tashalich Lake is one of the older sites (prior to 1900), the second greatest nucleotide diversity was found in the Vitus Lake resident freshwater population, and Vitus Lake is one of the youngest sites.

4.6 Discussion

Since the retreat of the Laurentide and Cordilleran Ice Sheets began approximately 20,000 ybp, both the ENAC and the TNPC fish expanded their ranges. It appears that Alaska and British Columbia represent a region of admixture between the two clades (Cresko, 2000). The presence of both clades in the same Bering populations supports this interpretation. Any potential for the two clades to differentiate across the species boundary appears to have lapsed when they came into contact.

Previous studies in North America failed to detect the presence of stickleback from the TNPC in marine or anadromous populations, though they commonly occur in resident freshwater populations (O'Reilly et al., 1993; Ortí et al., 1994). We too found

the TNPC to be common in resident freshwater populations, though we also found TNPC haplotypes among anadromous fish from the Tsiu River, Creek 1, Unnamed Lake, Tashalich Lake, Vitus Lake, and the Bering River lake site (Figure 4.2). Ortí et al. (1994) hypothesized that the lack of TNPC marine haplotypes in their study could be due to a small sample size coupled with a low frequency of occurrence in oceanic populations. The frequency of the TNPC haplotype in anadromous populations in the Bering Glacier region was greatest at the Bering River lake site (8.5%; Figure 4.2). The presence of the TNPC haplotype in Bering anadromous populations may be due to hybridization with TNPC resident freshwater fish; a low level of hybridization is consistent with the low frequency of intermediate lateral plate morphs in the species pair sites (von Hippel & Weigner, 2004).

All three resident freshwater populations that have a greater frequency of TNPC fish than ENAC fish occur in lakes outside of the 1900 Bering Glacier Neoglacial maximum (Figures 4.2 & 4.4), indicating that these three resident freshwater populations are older than many of the populations with a greater frequency of ENAC fish. These sites originally may have been colonized by TNPC fish, or a combination of clades. Given the low frequency of TNPC haplotypes in the extant anadromous populations, it appears that the frequencies of occurrence of the two haplotypes reversed, with the ENAC gaining prominence in newly created sites.

The levels of genetic diversity based on the NADH2 sequence varied minimally between populations and were generally low (Tables 4.3 & 4.4). The Tashalich Lake

resident freshwater population exhibited the greatest population differentiation, being significantly different from other Bering Glacier sites (Table 4.4). As the Tashalich Lake site is an older site (prior to 1900), it is not surprising that the resident freshwater stickleback have differentiated significantly from other populations (Figures 4.4, 4.5, & 4.6).

Resident freshwater populations display varying frequencies of TNPC fish, though all have a higher occurrence than is found in any anadromous population (Figure 4.2). This result supports the hypothesis that the species pairs formed by independent colonization of pre-existing resident freshwater and anadromous fish. If the resident freshwater populations arose by sympatric speciation or double invasion by anadromous fish following recent site formation, then their haplotype frequencies would be expected to be very similar to the anadromous populations.

NADH2 sequence divergence does not provide clear evidence for one origin pathway over another. The NADH2 sequence divergence of the Tashalich resident freshwater population supports independent colonization at this site, given that the NADH2 gene is a conserved region under selection and therefore would not be expected to diverge over a short period of time. However, it is not known how old this site is, and it may be thousands of years old (Molnia & Post, 1995). Vitus Lake, on the other hand, did not exist prior to 1900. The pairwise F_{ST} value of Vitus Lake resident freshwater and anadromous populations, based on NADH2 sequence, was not significant. This result could arise from any of the origin hypotheses.

The Bering Glacier region exists in a zone of admixture of the TNPC and ENAC haplotypes. It is also a region with both newly created and older freshwater sites in which species pairs are relatively common. These features make the region an excellent natural laboratory for the study of evolutionary divergence. A plethora of evidence supports the independent colonization hypothesis for the origin of the species pairs. The low frequency of intermediate phenotypes (von Hippel & Weigner, 2004; Chapter 2), the lack of co-variation in body shape between resident freshwater and anadromous forms (Chapter 2), the lack of correlation between body shape divergence and site age (Chapter 2), and the higher frequency of TNPC haplotypes among resident freshwater fish are all consistent with the independent colonization hypothesis. Future research should examine broader scale sequence divergence in both the mitochondrial and nuclear genomes in order to advance our understanding of contemporary evolution.

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Table 4.1. Threespine stickleback populations from the Bering Glacier region, locations in decimal degrees (datum WGS84), life history, and sample size. Differences in coordinate precision are due to the number of times the water body was sampled.

Site Name	Latitude (N)	Longitude (W)	Life History	N
Bering River Lake Site	60.36	143.973	Anadromous	48
			Resident Freshwater	34
Creek 1	60.103	143.36	Anadromous	48
			Resident Freshwater	31
East Tsivat Lake	60.13512	142.976	Resident Freshwater	47
Pond 1 (near Vitus Lake)	60.11587	143.2926	Resident Freshwater	48
Pond 2 (near Bering River)	60.455	144.208	Resident Freshwater	48
Tashalich Lake	60.039	143.63	Anadromous	31
			Resident Freshwater	48
Tsiu River	60.08	143.05	Anadromous	46
			Resident Freshwater	47
Tsivat Lake	60.16788	142.987	Anadromous	3
			Resident Freshwater	45
Unnamed Lake	60.102	143.55	Anadromous	48
			Resident Freshwater	48
Vitus Lake	60.1	143.2	Anadromous	42
			Resident Freshwater	47

Table 4.2. Pairwise F_{ST} values for 17 threespine stickleback populations from the Bering Glacier region, Alaska. Negative F_{ST} values in some cells are interpreted as a lack of genetic differentiation between the two populations and likely reflect the imprecision of the algorithm used by the software to estimate the value (Excoffier & Lischer, 2010). Asterisks represent significance after the application of the Bonferroni correction, *<0.05, **<0.01, and ***<0.001.

	Vitus Lake (A)	Tsiu River (A)	Tsivat Lake (A)	Tashalich Lake (A)	Unnamed Lake (A)	Creek 1 (A)	Bering River Lake Site (A)	Vitus Lake (FW)	Tsiu River (FW)	Tsivat Lake (FW)	Tashalich Lake (FW)	East Tsivat Lake	Pond 1	Pond 2	Unnamed Lake (FW)	Creek 1 (FW)	Bering River Lake Site (FW)
Vitus Lake (A)	-																
Tsiu River (A)	-0.02310	-															
Tsivat Lake (A)	-0.18060	-0.18247	-														
Tashalich Lake (A)	-0.02601	-0.02314	-0.17261	-													
Unnamed Lake (A)	-0.01240	-0.00826	-0.15033	-0.02468	-												
Creek 1 (A)	-0.02067	-0.01809	-0.16694	-0.02723	-0.01800	-											
Bering River Lake Site (A)	-0.01240	-0.00826	-0.15033	-0.02468	-0.02128	-0.01800	-										
Vitus Lake (FW)	0.15192	0.16456*	0.01762	0.11097	0.10057	0.13301	0.10057	-									
Tsiu River (FW)	0.28359***	0.29877***	0.14471	0.23416*	0.22989***	0.26647***	0.22989***	0.01468	-								
Tsivat Lake (FW)	0.69853***	0.71048***	0.61702	0.65796***	0.65467***	0.68520***	0.65467***	0.38879***	0.23490*	-							
Tashalich Lake (FW)	0.71080***	0.72209***	0.63801	0.67249***	0.66832***	0.69773***	0.66832***	0.40869***	0.25471***	-0.02142	-						
East Tsivat Lake	0.75566***	0.76571***	0.69807	0.72133***	0.71373***	0.74198***	0.71373***	0.46126***	0.30679*	-0.01349	-0.01669	-					
Pond 1	0.03055	0.03826	-0.09883	0.00585	-0.00180	0.01603	-0.00180	0.02972	0.13679	0.56509***	0.58158***	0.63003***	-				
Pond 2	0.12506	0.13681	-0.00699	0.08701	0.07599	0.10638	0.07599	-0.01965	0.03232	0.42326***	0.44269***	0.49456***	0.01304	-			
Unnamed Lake (FW)	0.29540***	0.31057***	0.15789	0.24593***	0.24228*	0.27867***	0.24228***	0.02176	-0.02118	0.22095	0.24051*	0.29203***	0.14860	0.04062	-		
Creek 1 (FW)	0.01539	0.02330	-0.11898	-0.00902	-0.01554	0.00052	-0.01554	0.03608	0.14360	0.57547***	0.59288***	0.64498***	-0.02606	0.01824	0.15519	-	
Bering River Lake Site (FW)	0.08889	0.10079	-0.05034	0.05114	0.03846	0.06813	0.03846	-0.01229	0.06258	0.47872***	0.49836***	0.55262***	-0.01278	-0.02017	0.07240	-0.01037	-

Table 4.3. Genetic diversity indices (mean and 1 standard error) for NADH2 mtDNA sequence data (π, nucleotide diversity; h, number of haplotypes; h_d, haplotype diversity) by population in the Bering Glacier region. The additional group mtDNA sequences were downloaded from Genbank and consist of a combination of a threespine stickleback (Accession Number NC_003174), a blackspotted stickleback (*Gasterosteus wheatlandi*, Accession Number NC_011570) and a ninespine stickleback (*Pungitius pungitius*, Accession Number NC_011571).

Population	N	Π	H	h_d
Tashalich Lake Anadromous	20	0.00634 (0.000742)	3	0.279
Tashalich Lake Freshwater	5	0.01436 (0.003207)	3	0.700
Vitus Lake Anadromous	14	0	1	0
Vitus Lake Freshwater	30	0.00831 (0.000511)	3	0.398
Additional Group	3	0.17949 (0.02967)	3	1.000

Table 4.4. Pairwise F_{ST} values for two species pairs from the Bering Glacier region and one additional group. The additional group mtDNA sequences were downloaded from Genbank and consist of a combination of a threespine stickleback (Accession Number NC_003174), a blackspotted stickleback (*Gasterosteus wheatlandi*, Accession Number NC_011570) and a ninespine stickleback (*Pungitius pungitius*, Accession Number NC_011571). Negative F_{ST} values in some cells are interpreted as a lack of genetic differentiation between the two populations and likely reflect the imprecision of the algorithm used by the software to estimate the value (Excoffier and Lischer 2010). Asterisks represent significance after the application of the Bonferroni correction, *<0.05, **<0.01, and ***<0.001.

Population	Vitus Lake Freshwater	Vitus Lake Anadromous	Tashalich Lake Freshwater	Tashalich Lake Anadromous	Additional Group
Vitus Lake Freshwater	-				
Vitus Lake Anadromous	0.04932	-			
Tashalich Lake Freshwater	0.58741*	0.85193**	-		
Tashalich Lake Anadromous	-0.03673	0.02381	0.64935**	-	
Additional Group	0.74516**	0.71525*	0.33018	0.71358**	-

Table 4.5. AMOVA between anadromous and resident freshwater populations in the Bering Glacier region.

Source of Variation	Df	Sum of Squares	Variance Components	Percentage of Variation
Between anadromous and freshwater	1	3.050	-0.206	-24.07
Among populations within groups	2	12.158	0.435	50.77
Within populations	65	40.850	0.628	73.30
Total	68	56.058	0.857	

Table 4.6. AMOVA between Vitus Lake and Tashalich Lake populations.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Between Vitus Lake and Tashalich Lake	1	2.164	-0.223	-26.52
Among populations within groups	2	13.044	0.435	51.76
Within populations	65	40.850	0.628	74.76
Total	68	56.058	0.841	

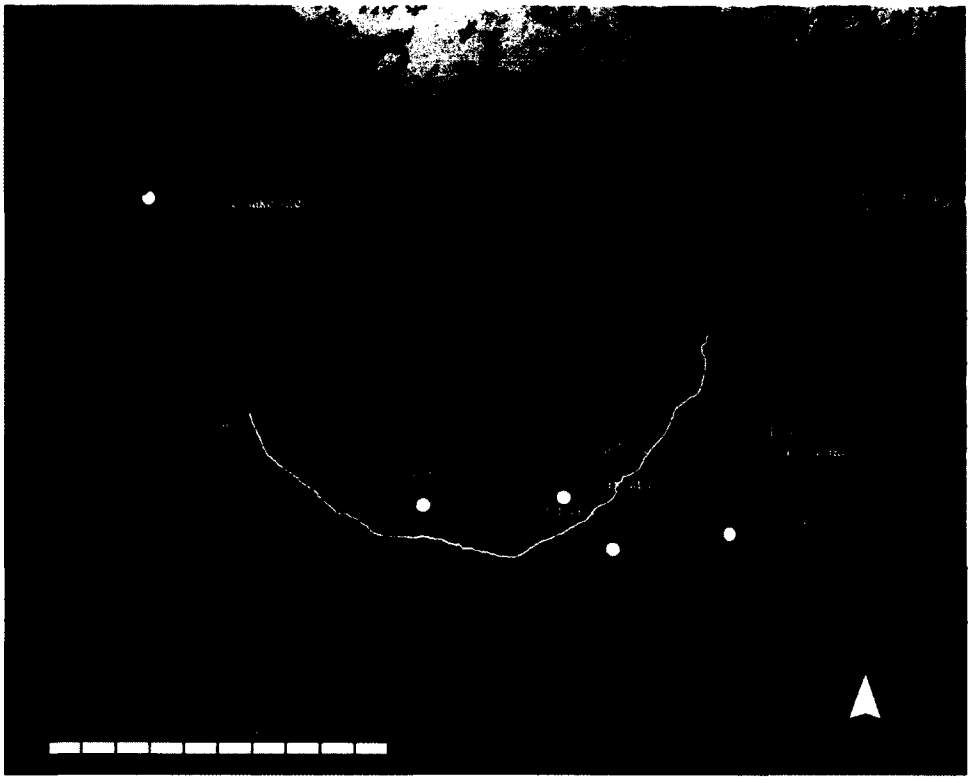

Figure 4.1. Landsat imagery of the Bering Glacier region, Alaska, showing all populations used in analyses. The white line represents the glacier's terminus at 1900. Note that Vitus Lake, Creek 1, Unnamed Lake, and Pond 1 fall within the ice-covered landscape of 1900, and hence are recently formed sites, while Tashalich Lake, Tsivat Lake, the Tsiu River, and East Tsivat Lake existed prior to 1900. Yellow circles represent species pairs used in the clade analysis, pink circles represent species pairs used in both the clade analysis and NADH2 analysis, and the aqua circles represent resident freshwater populations used in the clade analysis. The Landsat image was taken on September 10, 2001.

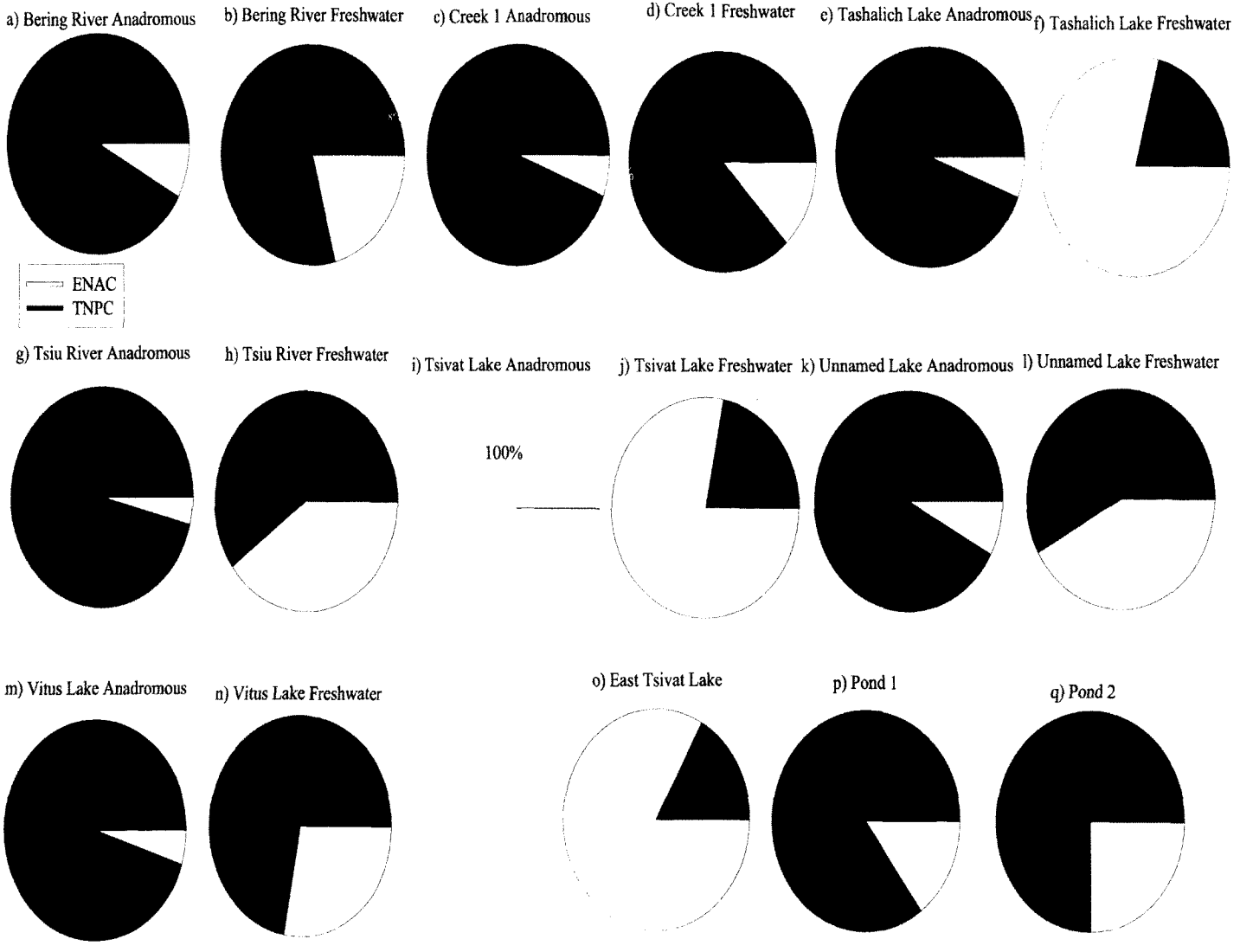

Figure 4.2. mtDNA haplotype frequencies for anadromous and resident freshwater threespine stickleback in the Bering Glacier region, Alaska. Black indicates the ENAC haplotype and white indicates the TNPC haplotype.

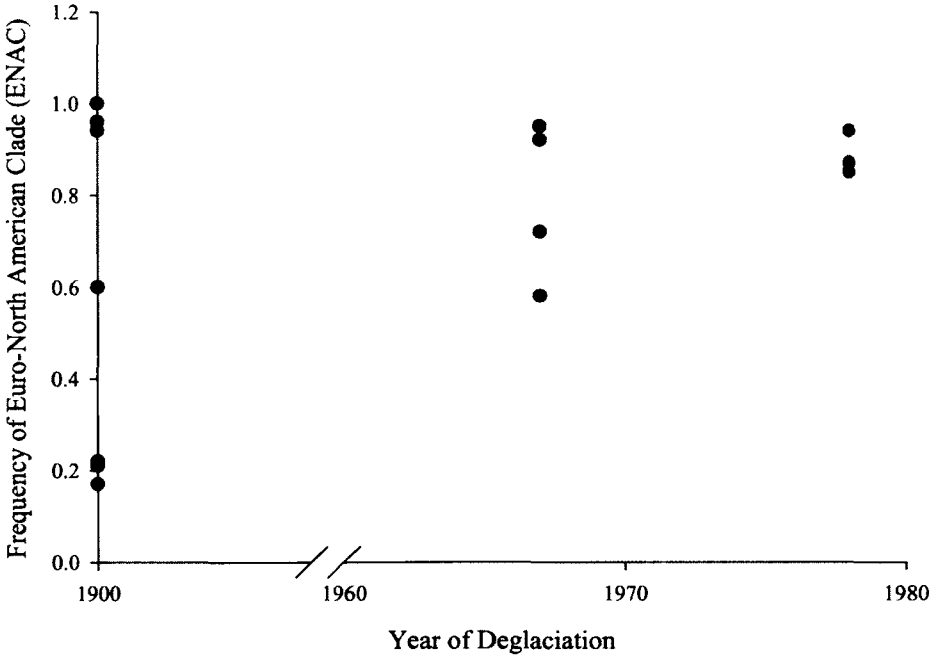

Figure 4.3. Frequency of ENAC fish plotted against the year of deglaciation.

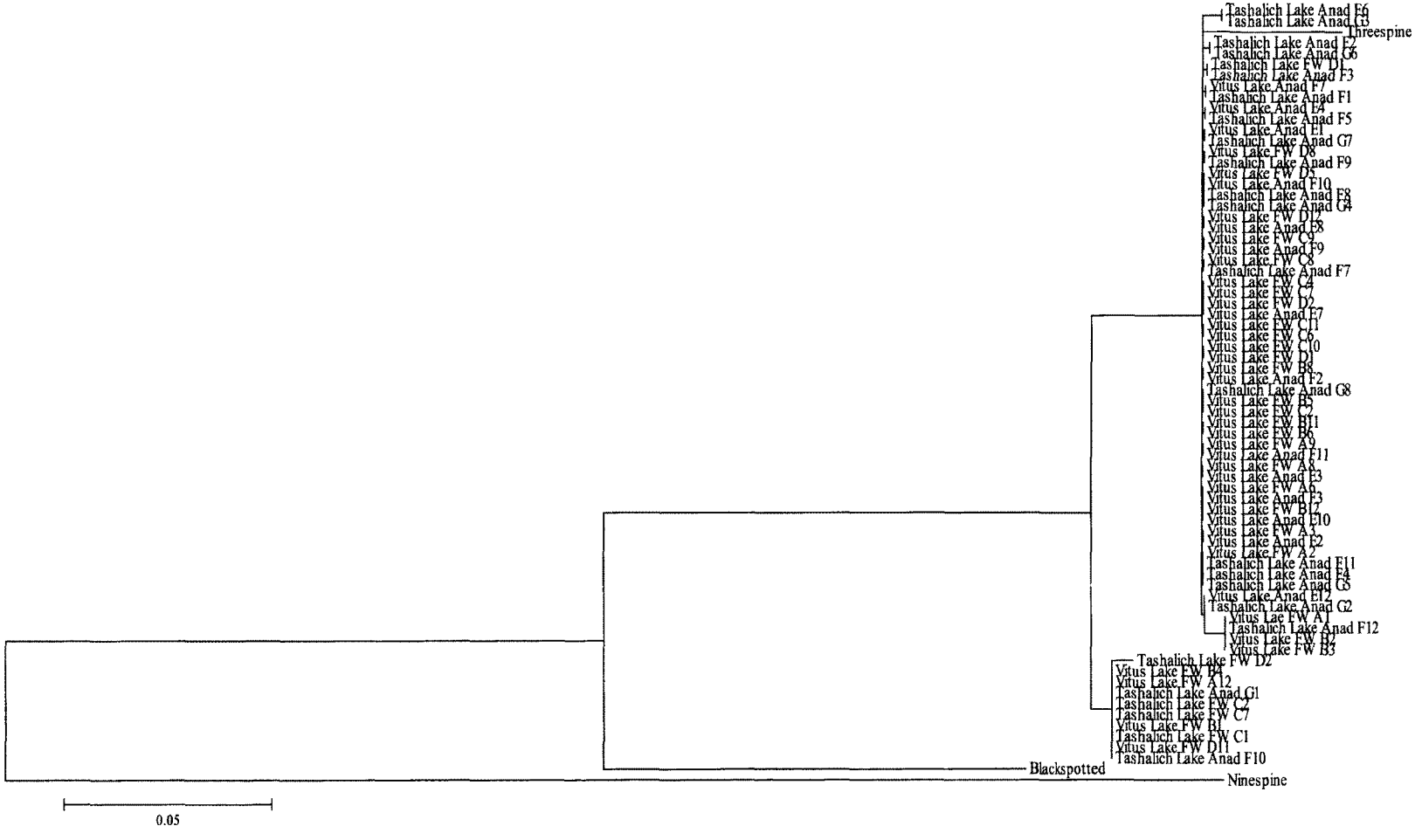

Figure 4.4. Maximum likelihood phylogenetic tree constructed from 72 NADH2 sequences based on the HKY+I model of nucleotide substitution.

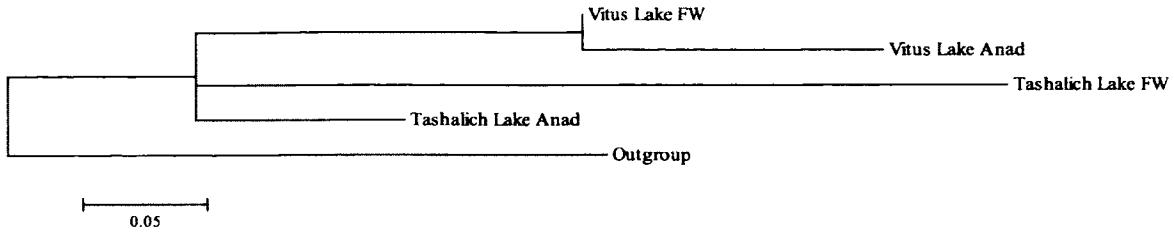

Figure 4.5. Minimum evolution phylogenetic tree constructed from γ_{ST} values.

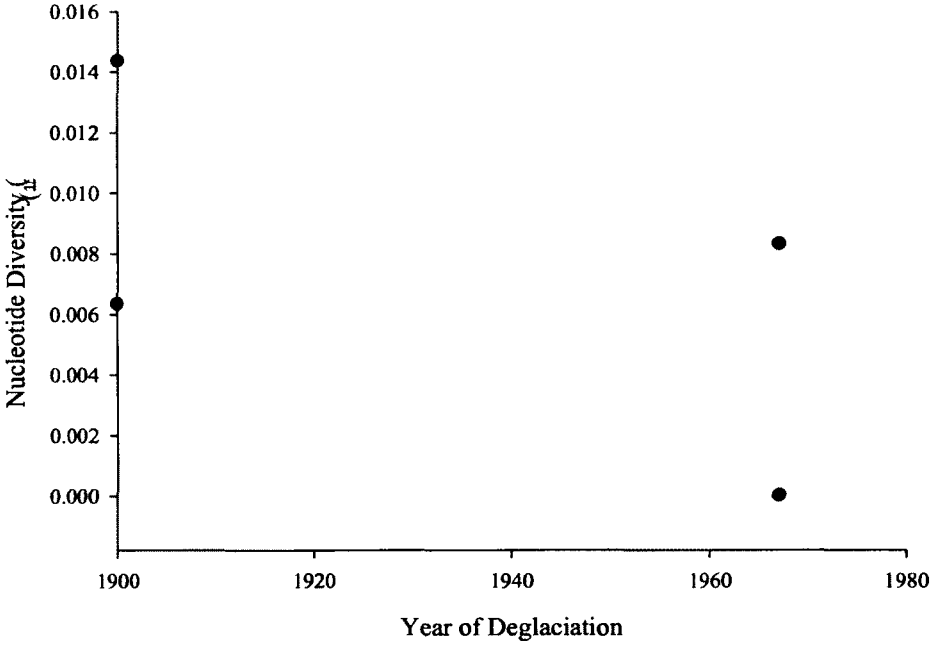

Figure 4.6. Nucleotide diversity plotted against the year of deglaciation.

Chapter 5 Conclusion

In the time since the retreat of the Laurentide and Cordilleran Ice Sheets began, approximately 20,000 ybp, limited progress into the understanding of the processes by which fish colonize newly deglaciated environments have been made. Previous work on newly deglaciated systems was conducted in Glacier Bay National Park, Alaska and limited to stream colonization by aquatic macroinvertebrates and salmonids (Milner, 1987; Milner & Bailey, 1989; Milner, 1994; Milner et al., 2000). The Bering Glacier began retreating from its Holocene maximum near the Gulf of Alaska around 1900; it provides a new landscape to study fish colonization and contemporary evolution.

5.1 Chapter 2

The purpose of this study was to determine fish species distribution throughout the Bering Glacier region. I related species presence to a variety of abiotic and biotic factors to provide aquatic baseline information to the U.S. Bureau of Land Management for management decisions. These lakes and streams are colonized from the ocean by diadromous species or from glacial refugia by resident freshwater species. Therefore, the potential pool of colonist species is relatively small.

Freshwater habitats around the Bering Glacier are both young and unstable as they experience periodic glacial surges and glacial outburst floods. As the ice free landscape around the Bering Glacier continues to change and expand with deglaciation, more stable aquatic landscapes and thus fish communities would be expected to form

(Evans et al., 1987). Habitat complexity promotes fish community development by providing diversified niches with a richer prey base and refuge from predators. The Bering Glacier Watershed is mostly young without much structural complexity to its aquatic habitats. As the glacier continues to recede, the watershed will likely evolve from ice contact, unstable lakes and streams that are over-run by glacial surges and outburst floods every 20-30 years to mature lakes and streams with a relatively stable hydrology. This, in turn, should lead to a more complex habitat characterized by aquatic vegetation, algae, woody debris and a diversified prey base. Local fish species richness (alpha diversity) will be likely to increase as a consequence.

A broad range of surface water qualities was found in the region, which is consistent with a diversity of habitat types from massive proglacial lakes to small isolated ponds. Not surprisingly, Bering Glacier fishes, most of which are early colonizing species and therefore adaptable to new environments, are found in a wide range of water qualities characteristic of habitat diversity.

Fish species that assume a resident freshwater life history and reproduce in isolated freshwater habitat, such as Dolly Varden, threespine stickleback and prickly sculpin, can readily adapt to the changing aquatic landscape. This likely explains why these species were found equally often in isolated and connected lakes and streams. As the stabilization of hydrological networks progresses with deglaciation, an increasing number of lakes and streams will likely become isolated due to the lack of seasonal

connections from glacial meltwater and the establishment of regular stream channels that are no longer influenced by ice dams that alter drainages. This should further sort the species so that only those able to evolve into resident freshwater forms are found in isolated fresh waters.

Dolly Varden was the only species that was more likely to be found in glacial lakes and streams. Previous studies in Glacier Bay National Park showed that Dolly Varden are able to thrive in the harsh environment of glacial streams and lakes because they require less habitat complexity than other fishes, such as coho salmon (Milner & Bailey, 1989); furthermore, Dolly Varden are the first of the salmonids to colonize new stream habitat (Milner, 1994).

The fish species present in these newly formed lakes and streams are typical for the Alaskan coastal region; however, some species have evolved in atypical ways (i.e. – threespine stickleback species pairs and dwarfism in Dolly Varden).

Given the uniqueness of species pairs of threespine stickleback, they warrant further study. I proposed three hypotheses as to their origin: sympatric speciation of anadromous stickleback, double invasion of anadromous ancestors from the Gulf of Alaska, or independent colonization from a previously existing resident freshwater population in addition to colonization by an anadromous population from the Gulf of Alaska (von Hippel & Weigner, 2004; Appendix A). I employed the use of geometric morphometrics (Chapter 3) and genetic analyses (Chapter 4) to address these hypotheses.

5.2 Chapter 3

Geometric morphometric analyses were employed to determine if body shape differences exist between anadromous and resident freshwater members of species pairs. This is important because most of our study sites formed due to glacial melt after 1900 and some sites formed much more recently than that (von Hippel & Weigner, 2004; Weigner & von Hippel, 2010). If resident freshwater fish evolved from anadromous fish recently following site formation, then we would expect significant association of body shapes indicating that the ancestral anadromous form and the derived resident freshwater form were evolving along the same trajectory. The lack of an association in body shape between members of a species pair suggests that divergence between them was not recent (i.e. since sites formed in this newly deglaciated region). Furthermore, the lack of correlation between divergence in geometric morphometric variables and year of deglaciation also suggests non-recent divergence. Therefore, these body shape results suggest that the species pairs did not evolve by sympatric speciation or by double invasion of anadromous fish following recent site formation; rather, they likely formed due to independent colonization by pre-existing anadromous and resident freshwater populations into newly created freshwater sites (von Hippel & Weigner, 2004).

5.3 Chapter 4

Genetic analyses were employed in an attempt to validate the geometric morophometric conclusions. I first analyzed the frequency of the ENAC vs. the TNPC

based on the cytochrome b gene on the mtDNA genome. Resident freshwater populations display varying frequencies of TNPC fish, though all have a higher occurrence than is found in any anadromous population. This result supports the hypothesis that the species pairs formed by independent colonization of pre-existing resident freshwater and anadromous fish. If the resident freshwater populations arose by sympatric speciation or double invasion by anadromous fish following recent site formation, then their haplotype frequencies would be expected to be very similar to the anadromous populations.

I also sequenced a portion of the NADH2 gene on the mtDNA genome. The levels of genetic diversity based on the NADH2 sequence varied minimally between populations and were generally low. The Tashalich Lake resident freshwater population exhibited the greatest population differentiation, being significantly different from all other Bering Glacier sites. As the Tashalich Lake site is an older site (prior to 1900), it is not surprising that the resident freshwater stickleback have differentiated significantly from other populations. NADH2 sequence divergence does not provide clear evidence for one origin pathway over another. The NADH2 sequence divergence of the Tashalich resident freshwater population supports independent colonization at this site, given that the NADH2 gene is a conserved region under selection and therefore would not be expected to diverge over a short period of time. However, it is not known how old this site is, and it may be thousands of years old (Molnia & Post, 1995). Vitus Lake, on the

other hand, did not exist prior to 1900. The pairwise F_{ST} value of Vitus Lake resident freshwater and anadromous populations, based on NADH2 sequence, was not significant. This result could arise from any of the origin hypotheses.

5.4 Future Work

A thorough sampling of the streams in late summer and fall should be performed to determine which salmon species are present and the strength of their runs. Sampling in the spring when salmon emerge from their redds would provide additional information on population dynamics. For example, pink and chum salmon go to sea immediately after emergence from redds (McPhail & Lindsey, 1970), and hence may have been missed in our sampling. The habitats of current and potential salmon streams need to be protected from pollution events or other causes of habitat degradation.

As the Bering Glacier continues to surge and retreat, it provides the opportunity to study contemporary evolution in real time. The populations of dwarf Dolly Varden in the Berg Lake and Khitrov Lake areas are isolated by massive waterfalls and glacial ice, respectively. These and other Dolly Varden populations are unusual, and deserve further study of their life history and phylogeography. It is not understood if these populations are dwarfed due to environmental constraints, such as poor nutrition, high density, and elevated pH, and/or due to genetic factors, such as a mutation in the insulin-like growth factor 1 allele or allele fixation due to the founder effect, genetic drift, inbreeding, or selection. Dwarf populations of Dolly Varden also occur in the Matanuska-Susitna

Valley (Bell, pers. comm.) and the Bristol Bay region (McPhail & Lindsey, 1970) of Alaska, but these locations deglaciated thousands of years ago, while many of the Bering Glacier sites are less than 100 years old.

Since the Berg Lake and Khitrov Lake fish live above dispersal barriers, a phylogenetic analysis may provide interesting insights into how Dolly Varden colonized such isolated habitat. Possibilities include delivery by piscivorous birds who bring live prey to their young (M. S. Christy, pers. comm.) or dispersal through glacial conduits. Such unusual methods of colonization would likely be reflected in low genetic diversity due to a population bottleneck. Phylogenetic relationships with surrounding populations may yield the source population(s) of the colonists. Furthermore, the question arises as to whether Dolly Varden became dwarfed in these young sites or if they colonized these sites while already dwarfed. Based on genetic analyses, if these Dolly Varden populations prove to be highly differentiated from other populations around the state, then they may warrant special consideration for conservation.

As we learn more about the fishes of the Bering Glacier Watershed, we may find additional, unusual populations. It is clearly a watershed of paramount importance to understanding colonization, succession and evolution of aquatic organisms in newly deglaciated terrain.

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Appendix A Species pairs of threespine stickleback in new lakes and streams at the Bering Glacier, Alaska

Summary

1. Resident freshwater and anadromous pairs of threespine stickleback species (Gasterosteus aculeatus species complex) have been reported from only a few lakes.

2. We discovered such pairs in four lakes and four streams, including recently deglaciated terrain, near the Bering Glacier, Alaska.

3. Bering Glacier surges are accompanied and ended by massive glacial outburst floods, which create unmatched dynamics in the surrounding lakes and streams.

4. This is the first report of a stickleback species pair in a proglacial lake (Vitus), which did not exist 100 years ago.

5. These species pairs may have arisen through sympatric speciation, double invasion by anadromous stickleback, or independent colonization by resident and anadromous forms.

Prepared in the format for Behaviour. Published as: von Hippel, F. A. and H. Weigner. (2004) Species pairs of threespine stickleback in new lakes and streams at the Bering Glacier, Alaska. Behaviour 141: 1441-1464.

Introduction

Species pairs of threespine stickleback (Gasterosteus aculeatus species complex) with parapatric or sympatric ranges occur in six varieties (Table A – 1; reviewed by McPhail 1994, McKinnon & Rundle 2002). Of these, only the resident freshwater and anadromous pair has a broad geographical distribution. All other types have restricted ranges, suggesting that peculiarities of local geology and ecology and possibly stochastic events gave rise to them. For example, the benthic-limnetic pairs are thought to have arisen 11,000 years ago as the result of marine stickleback being trapped in freshwater due to isostatic rebound following the melting of Pleistocene ice (McPhail 1993, 1994). These fish would then be subject to strong directional selection, probably towards a benthic ecology. It is thought that when the lakes were reopened to colonization, possibly 9,000 years ago, a second invasion of marine stickleback evolved to exploit the limnetic niche. Similarly, local geological history may explain the origin of the Sea of Japan species pair (Higuchi & Goto 1996, Yamada et al. 2001). Conversely, the resident-anadromous pairs have originated independently and often, throughout the stickleback's range, constituting numerous replicates with different histories and local ecologies. These pairs therefore provide a unique opportunity to study the forces that drive parallel evolution and speciation.

The ecology of lakes differs greatly from that of streams, and therefore the lake-dwelling resident-anadromous pairs should be considered separately from the stream-dwelling pairs. Stream pairs are common in lowland streams throughout much of the

stickleback's range, including coastline areas of Europe, Asia and North America (McPhail 1994). Lake pairs, on the other hand, have been reported only from the Azabachije Lake basin in Kamchatka (Ziuganov et al. 1987), in Lake Sana on Iturup Island in the Kuril Islands (discussed in Mori 1990), in Lakes Harutori, Akkeshi and Hyotan on Hokkaido Island (Mori 1990, Higuchi et al. 1996, Kitano et al. 2003), and in Mud Lake in southcentral Alaska (Karve 2004). In this paper, we report eight additional resident-anadromous pairs of threespine stickleback species, including four lake pairs, from the drainage of the Bering Glacier, Alaska.

We report morphological results for four pairs (two lake and two stream) through samples collected in 2002 and 2003. One pair is in proglacial (ice-contact) Vitus Lake. This is the first discovery of a stickleback species pair in a proglacial lake. We also report the location of four additional species pairs discovered in 2003 and 2004 (Fig. A – 1, Table A – 2). All of these species pairs exist in areas just above sea level. Vitus Lake was completely covered with ice during the Little Ice Age local maximum around 1900 (Fig. A – 1). Given that Vitus Lake did not exist 100 years ago, and that glacial surges have caused massive disturbances including glacial outburst floods as recently as August 1994 and September 1995 (Fatland & Lingle 1998), the species pair has existed in this location for less than 100 years, and possibly only since some time after the 1995 flood.

The youth of the Vitus Lake species pair raises interesting questions about the speed and mechanisms by which sympatric fish pairs can form as new aquatic habitat is created in the path of receding glaciers. Research on the speciation process is hindered

by the fact that speciation is typically identified only after it has occurred (McPhail 1994). Incipient species, therefore, are the most useful for understanding genetic, ecological and behavioral processes leading to new species (McPhail 1994, Johannesson 2001). Previous work on species pairs of stickleback has sought to explain the origin of these pairs many thousands of years ago. At the Bering Glacier we have the opportunity to study the formation of species pairs in real time or shortly after their origin. In this paper we demonstrate that the species pairs are real and we hypothesize pathways to their origin and maintenance.

Ecological Instability of Aquatic Habitats below Bering Glacier

Bering Glacier is the largest and longest glacier outside of Greenland and Antarctica (Molnia & Post 1995, Molnia et al. 1996, Jaeger & Nittrouer 1999). It began to recede from near its late Holocene maximum at the start of the 20th century, creating several large proglacial lakes. The largest of these is Vitus Lake (25 km long, 10 km wide, to 180 m deep, 160 km^2 in area), which varies in size depending on glacial dynamics (Brouwers & Forester 1993, Muller & Fleisher 1995, Molnia et al. 1996). With the lake surface at ~2 m elevation (Brouwers & Forester 1993), most of the lake's water lies below sea level. Vitus Lake has changed markedly during its brief life from a small freshwater lake to a large system with tidal exchange through the Seal River. It now has a thin freshwater to brackish layer maintained by ice melt on top of a saline extension of the Gulf of Alaska (Brouwers & Forester 1993).

Bering Glacier surges every 20-30 years at the expense of upstream ice thickness (~1900, ~1920, ~1938-1940, 1957-1960, 1965-1967, 1993-1995) and is the Earth's largest surging glacier (Meier & Post 1969, Molnia & Post 1995). The 1938-1940 surge occupied nearly all of Vitus Lake. By 1948 the lake had become 10 km^2 in area, but was mostly reoccupied with ice in the 1957-1960 surge, resulting in a 3 km^2 lake (Molnia & Post 1995). Most of the existing lake has formed since 1967 (Fig. A – 1) by glacial recession of > 2 km some years (Molnia & Post 1995, Molnia et al. 1996). The latest surge (summer 1993 – summer 1995), which included glacial advances of 100 m per day (Roush 1996) and a 1,500 m advance in 17 days (Herzfeld & Mayer 1997), consumed about 2/3 of Vitus Lake (90% of the remaining lake was covered by icebergs) and reoccupied 16,734 ha of non-ice landcover before the glacier retreated again (Gray et al. 1994, Payne et al. 1997, Fig. A – 1). Part of the glacier's terminus was displaced downstream by about 9 km, partially or completely covering every island in Vitus Lake (Molnia 1995).

The surges are accompanied and ended by massive glacial outburst floods (jökulhlaups) originating from a subglacial conduit system, which, together with the rapid advances and retreats of the glacier, create unmatched dynamics in the surrounding aquatic habitats (Fleisher et al. 1998; Jaeger & Nittrouer 1999). The outburst flood of July 27-August 9, 1994 drastically altered proglacial topography (Merrand & Hallet 1996, Fleisher at al. 1998) and caused the loss of red (sockeye) salmon (Oncorhynchus nerka) and silver (coho) salmon (Oncorhynchus kisutch) runs in the Seal River. Surges

dramatically alter lake bathymetry, turbidity, and water temperature (Fleisher et al. 1998), as well as suspended sediment discharge through the Seal River (Gray et al. 1994, Merrand et al. 1994).

The dynamic nature of the proglacial lakes is not limited to episodes of surging. For example, a quarter century of glacial retreat before the latest surge expanded the width of Vitus Lake more than threefold. Glacial retreat also allowed water to break out of Tsiu Lake on August 10-11, 1989 and draw down both Tsiu Lake and Tsivat Lake 17 m when the water discharged into Vitus Lake and abandoned its former outlet over the course of a single week (Fleisher et al. 1993, Fleisher et al. 1998, see Fig. A – 1). This created a massive turbid plume in Vitus Lake that was visible for years. The 1993 surge closed the new outlet, quickly raised the Tsiu lake level 14 m, and forced drainage once again through the former outlet. The sedimentation rate from glacial meltwater and icebergs dropping their load is in the range of meters per year (Brouwers & Forester 1993, Molnia et al. 1996). Additionally, subglacial supercooled water (-0.15 to –1.0° C) is discharged through basal conduits into the proglacial lakes (Fleisher et al. 1993, Fleisher et al. 1998).

Methods

Field Sites and Morphological Variables

The Bering Glacier is accessible only by air. The U.S. Bureau of Land Management maintains a seasonal field station on the south shore of Vitus Lake. Sampling sites on Vitus Lake, Creeks 1 and 2, and Unnamed Lake were reached from the

field station by foot, all terrain vehicle and inflatable power boat. Midtimber Lake was reached by foot and helicopter, and the Tsiu River and wetlands, the Bering River, Tashalich River and numerous other sites were reached by helicopter.

Fish were trapped July 13-30, 2002, May 5-8, June 2-5, and July 16-August 15, 2003, and May 10-12, June 1-4, June 20-July 5, July 17-August 9, 2004 using 0.32 cm mesh unbaited minnow traps and unbaited semi-oval traps. Stickleback were killed with an overdose of MS-222 anesthetic, fixed in 10% buffered formalin, preserved in 70% ethanol, and stained with Alizarin red S.

Morphological traits were analyzed for the Vitus, Midtimber, Tsiu and Creek 1 species pairs collected in 2002 and 2003. Only fish > 32 mm standard length (SL) were scored because lateral plate development is complete in most threespine stickleback populations by this size (Bell 2001). The following traits were measured using digital calipers: SL, lengths of left and right pelvic spine and second dorsal spine, distance from end of dorsal fin to end of hyperal plate, length of base of dorsal fin, number of dorsal fin rays, body depth at second dorsal spine, length of snout, operculum width, orbit width, and length of left pectoral fin. The following traits were also scored under a dissecting microscope: sex, plate morph (low, partial, or complete), number of lateral plates on left and right side, number of gaps in plates on left and right side, left and right side pelvic score (1-4 on each side, Bell et al. 1993, Bell & Ortí 1994), and number of gill rakers on first right arch. Due to ongoing project refinement, not all traits were scored in 2002 for

the Vitus Lake fish and the number of dorsal fin rays and operculum width were only scored for 2003 samples.

Statistical Analyses

The SPSS statistical package v 11.5.5 was used for all statistical analyses. Because our morphological variables included both morphometric and meristic traits that we wished to analyze together, we z-transformed all traits so that each would have a mean of 0 and a standard deviation of 1 (Kachigan 1986). Fish were then subjected to a cluster analysis based on all z-transformed morphological variables in order to create a statistical division of fish into clusters. These clusters correspond to the resident and anadromous forms. Our sample sizes for Vitus (2002), Midtimber and Creek 1 were too small to subject to cluster analyses, so for these samples we relied on a priori visual classification as resident or anadromous. Our visual classification scheme was then compared to the results of the cluster analysis for the larger samples to ensure consistency of categorization. This strategy revealed that of the 815 fish subjected to the three cluster analyses (Vitus 2003 sample, Tsiu 2002 and 2003 samples), visual classification and cluster analysis provided inconsistent results with only 8 fish (1%).

Residuals were then calculated from the z-transformed values for all length variables separately for resident and anadromous fish, and the slopes and y-intercepts determined separately for the two groups of fish. This was repeated for each location each year. The y-value for each variable was calculated from the regression lines at the

mean SL of all fish from that location in that year (mean of resident + anadromous fish). The residuals were then adjusted by adding the y-values for each variable separately at the mean SL. This procedure caused each fish to be transformed into a fish with a SL equal to the mean SL of all fish for that location in that year, thereby removing any bias due to variation in SL. Following this, a MANOVA (Vitus 2003, Tsiu 2002, Tsiu 2003) or Mann-Whitney U test (because of small sample sizes; Vitus 2002, Creek 1) was run using the same size-adjusted residuals (for length traits) and z-transformed values (for meristic traits) to identify any mean differences between anadromous and resident fish in these traits (Kachigan 1986). Traits with no variance (plate morph, number of gaps in plates, pelvic score) or largely unequal variance (plate number) were excluded from the MANOVA but are reported in tables. All tests are two-tailed.

We used the 2003 samples of anadromous fish from Vitus and Tsiu and resident fish from Vitus, Midtimber and Tsiu to compare the same form of fish across sites. The Midtimber anadromous and Creek 1 samples were too small to include in multivariate analyses. Because MANOVAs are sensitive to large differences in sample size, the larger samples were randomly subsampled to equal the smallest anadromous or resident sample. We size-adjusted all anadromous fish to equal the mean anadromous SL and all resident fish to equal the mean resident SL, and then adjusted the residuals accordingly. For example, in comparing the anadromous fish from Vitus and Tsiu, all individuals were size adjusted to equal the mean SL of anadromous fish from both sites. In one MANOVA we included three traits associated with predator defense (adjusted residuals

of dorsal spine length, body depth, and total pelvic spine length, which together determine the minimum gape necessary for a successful piscivore) and two traits related to swimming performance (adjusted residuals of the distance between the dorsal fin and hyperal plate and width of the pectoral fin). In the second MANOVA we included four traits associated with trophic ecology (adjusted residuals of operculum width, snout length, orbit width and z-transformed number of gill rakers) as well as z-transformed number of dorsal fin rays. Of course all of these traits are associated with multiple functions (e.g., eye size is important for both predator avoidance and food acquisition), but the purpose was to conduct MANOVAs with a ratio of at least ten fish per dependent variable while still maintaining the traits in meaningful groups.

Results

We sampled a total of 44 lakes and 31 streams in the Bering Glacier region between 2002-2004. Of these, four lakes (9 %) and four streams (13 %) had both resident and anadromous stickleback (Table A – 2).

All of the Vitus Lake anadromous fish were complete morphs (score of 3), while the resident sample contained lows, partials and completes (mean of 2.0; completes had a caudal peduncle keel). Resident and anadromous fish differed significantly in expected ways in both 2002 and 2003 samples (Tables A – 3 & A – 4). The 2003 division of Vitus Lake fish into anadromous and resident forms, based on a cluster analysis, was supported by the MANOVA (multivariate $F_{(11, 98)} = 43.42$, $p < 0.001$).

Out of 404 fish from Midtimber samples collected in 2002 and 2003, only three were scored as anadromous, and therefore this population was not analyzed statistically. The three anadromous fish were complete morphs, while the resident sample contained lows, partials and completes (2002 mean of 1.9, 2003 mean of 1.7; completes had a caudal peduncle keel). The anadromous fish differed in other expected ways, such as having 23 gill rakers on the first right arch versus a mean of 19.8 in both the 2002 and 2003 resident samples.

All of the Tsiu anadromous fish were complete morphs (score of 3), while the resident sample contained all lows with the exception of two partials in 2002 and 5 partials and one complete in 2003. Resident and anadromous fish differed significantly in expected ways in both 2002 and 2003 samples (Tables A – 5 & A – 6). The 2002 and 2003 divisions of Tsiu fish into anadromous and resident forms, based on cluster analyses, were supported by the MANOVAs (2002 multivariate $F_{(9, 271)} = 481.50$, $p < 0.001$; 2003 multivariate $F_{(11, 376)} = 774.33$, $p < 0.001$).

All of the Creek 1 anadromous fish were complete morphs, while the resident sample contained lows, partials and completes (Table A – 7). Resident and anadromous fish differed significantly in expected ways.

A MANOVA testing for differences between anadromous fish in Vitus and Tsiu for traits largely associated with predator defense (see Methods) revealed a significant effect of location (multivariate $F_{(5, 83)} = 42.59$, $p < 0.001$) which included significant univariate effects of all five traits: dorsal spine length ($F_{(1, 87)} = 34.32$, $p < 0.001$), body

depth ($F_{(1, 87)} = 36.83$, $p < 0.001$), total pelvic spine length ($F_{(1, 87)} = 53.45$, $p < 0.001$), distance between dorsal fin and hyperal plate ($F_{(1, 87)} = 36.17$, $p < 0.001$), and width of pectoral fin ($F_{(1, 87)} = 78.50$, $p < 0.001$). A MANOVA comparing anadromous fish for traits largely associated with trophic ecology also revealed a significant effect of location (multivariate $F_{(5, 87)} = 13.71$, $p < 0.001$) which included significant univariate effects of three of the five traits: operculum width ($F_{(1, 91)} = 37.95$, $p < 0.001$), snout length ($F_{(1, 91)} = 21.99$, $p < 0.001$) and orbit width ($F_{(1, 91)} = 47.44$, $p < 0.001$). MANOVAs comparing resident fish between Vitus, Tsiu and Midtimber for both defense traits and trophic traits also revealed significant effects of location (Table A – 8).

Discussion

Lacustrine pairs of resident and anadromous threespine stickleback species have been found only in a few lakes that drain into the North Pacific (Ziuganov et al. 1987, Mori 1990, Higuchi et al. 1996, Kitano et al. 2003, Karve 2004). The results of this study demonstrate that such pairs also exist in relatively young lakes at the Bering Glacier (Table A – 2), also part of the North Pacific drainage. Most of Alaska, Russia, and islands in the North Pacific and North Atlantic are roadless and remote and therefore have not been sampled for stickleback. Pairs of resident and anadromous stickleback may be reasonably common in lakes throughout the northern part of their range, particularly in newly formed lakes left behind by receding glaciers.

The presence of a species pair in proglacial Vitus Lake demonstrates that substantial ecological and evolutionary interactions are occurring in low productivity proglacial lakes. Robinson and Wilson (1994) found that divergence in morphological traits is more likely to occur when fish are in species-poor communities. Such communities should have reduced predatory and competitive pressures allowing more variants to survive and diverge (McPhail 1994). This might explain the observation that species pairs of fishes occur most often in recently glaciated landscapes, which have depauperate fish faunas (McPhail 1994). In the lakes around Bering Glacier it may have more to do with the low density of fish leading to reduced competition and predation, rather than the low species richness (Weigner & von Hippel unpublished data). The low density, in turn, is probably due to the low productivity in such new habitat and the massive abiotic disturbances that repeatedly 'reset' the ecological clock. The colonization and evolution of fish in proglacial lakes has seldom been studied, but these lakes have the very conditions, such as reduced competition and predation, that should promote the formation of species pairs. The understanding of fish evolution in these ice-contact systems awaits their exploration.

Morphological differences between the anadromous and resident forms at the Bering Glacier are typical of these species pairs. Anadromous stickleback are large, completely-plated and have about 23 gill rakers per arch, and resident fish are small, have variable plate morphology and about 19-20 gill rakers per arch (Tables A – 3 – A – 7). Anadromous and resident forms differ in body size, shape, foraging traits, armor, nuptial

coloration and courtship behavior, a suite of traits subject to both natural and sexual selection (reviewed by McPhail 1994, McKinnon & Rundle 2002). The greater number of gill rakers and larger eyes of the anadromous form in the Bering Glacier area are consistent with a more planktivorous diet, as expected (McPhail 1994).

It is common for resident populations to differ morphologically from each other, and this was also shown to be the case in the comparisons between the Vitus, Midtimber and Tsiu resident forms. These resident forms appear to represent independently evolving isolates. However, it is usually assumed that anadromous stickleback come from a single, panmictic population and therefore vary little between sites. Our results of significant morphological differences between the Vitus and Tsiu anadromous forms suggest that interpopulation variation and homing behavior in anadromous stickleback is well worth further investigation.

While we do not know how long it has been since the resident populations were derived from anadromous ancestors, the Vitus Lake species pair, as well as the nearby pairs in Creek 1, Creek 2 and Unnamed Lake, is quite young. At the start of the 20th Century, the Bering Glacier terminated in an outwash plain, and Vitus Lake did not exist (Molnia & Post 1995; Fig. A – 1). Vitus Lake was massively disturbed by the surging Bering Glacier and glacial outburst floods as recently as 1965-1967 and 1993-1995. Therefore, the Vitus Lake species pair is younger than 100 years old, and may have formed after 1967 or 1995 (although they may well have persisted through these floods). The other four species pairs (Table A – 2) may also be quite young. The youth of these

species pairs and their formation in a proglacial lake (Vitus) and nearby, formerly proglacial lakes and streams in constant states of flux raise the question of their origin and maintenance.

Origin of Species Pairs

Explanations for the origin of species pairs in British Columbia and elsewhere often rely on geological events, such as isostatic rebound, that take hundreds or thousands of years to complete. Glacial retreat in Alaska suggests, however, that the retreat of the ice sheets elsewhere about 11,000 years ago was not a simple process, and that catastrophic events such as surges and glacial outburst floods may have played an integral role in the (rapid?) formation of these better known species pairs.

At least three pathways may have given rise to Bering Glacier species pairs: sympatric speciation from an anadromous ancestor, double invasion of anadromous stickleback, or colonization by resident stickleback from adjacent areas either before or after colonization by anadromous stickleback from the sea. Sympatric speciation is an unlikely explanation for all sites, and double invasion may only be feasible in Midtimber Lake. For all other sites, independent colonization appears to be the most likely explanation for the origin of species pairs.

Sympatric Speciation Hypothesis

For sympatric speciation to occur, some offspring of anadromous fish must fail to return to the sea, and mate assortatively with each other, while anadromy was retained by other fish from the same population. This might occur through major selection differentials acting on opposite sides of an ecotone (McPhail 1994), but such disruptive selection seems unlikely in the Bering Glacier area where the locations of ecotones constantly shift due to abiotic disturbance. Sympatric speciation has also been discounted in other species pairs (McPhail 1994).

Double Invasion Hypothesis

Double invasion could be mediated by the local geomorphology of Midtimber Lake. Midtimber Lake was formerly a high velocity ice-marginal river that drained Bering Glacier (Molnia & Post 1995). Between 1920-1938, Midtimber River separated from the glacier as Vitus Lake captured the discharge that had previously flowed into Midtimber (Molnia & Post 1995). By the early 1950s, Vitus Lake drained entirely through the Seal River, and Midtimber became tidally dominated and collected meltwater from stagnant ice remaining from the 1967 surge (Molnia & Post 1995, B. Molnia pers. comm.). The river became a lake that has continually shrunk. Since the early 1970s winter wave action in the Gulf of Alaska has often closed the outlet with sand, and Midtimber's outlet opens only occasionally after heavy rains (B. Molnia pers. comm.). Most recently, we found the outlet opened from heavy rains in the latter half of August, 2003. Due to these

typical outlet blockages, Midtimber Lake has had opportunities for a double invasion. Freshwater forms of fish often evolve after sea-run fish become land-locked by an extrinsic barrier (McDowall 1988).

This typical outlet blockage could explain why the proportion of anadromous fish seems to be so low and the frequency of intermediate phenotypes high in Midtimber Lake. If anadromous fish only occasionally enter Midtimber, and then become trapped, directional selection would continually force their evolution towards a resident freshwater phenotype. Additionally, the low frequency of anadromous fish in Midtimber may lead to gene flow because of the greater difficulty imposed on the rare anadromous fish in finding an anadromous mate. In particular, anadromous females may be likely to mate with resident males (Furin & von Hippel unpublished data). The regular (perhaps frequent) influx of anadromous fish during years when the outlet is open would lead to the variable distribution that we see in the morphology of the Midtimber fish, either through selection and/or gene flow. Midtimber Lake may have a leaky species pair, with gene flow from anadromous fish continually eroding divergence towards a resident phenotype.

Outlet blockages leading to double invasion may only persist for some years until excessive meltwater and precipitation breach the dunes, so this double-invasion model requires that trapped fish evolve quickly enough to favor assortative mating after, perhaps, less than a decade of isolation. Results from several populations of threespine stickleback indicate that the rate of evolution in freshwater isolates can be rapid enough

to cause appreciable morphological change in just one decade (Bell 2001). For example, Loberg Lake stickleback evolved from a nearly monomorphic (96%) complete morph population in 1990 to a low-morph dominated population in 2001 (75% low, 11% complete) and during this time gill raker number and body form also evolved rapidly (Aguirre 2003, Bell et al. in press). Moreover, reproductive isolation evolved between low and complete morphs in the White Sea basin within eight generations of introduction to a pond (Ziuganov 1995). Since body size is an important cue for assortative mating (McPhail & Hay 1983, Borland 1986, Nagel & Schluter 1998, McKinnon et al. 2004), and the F1 of anadromous stickleback raised in freshwater are smaller than their parents (Mori 1990), nearly instant isolation may occur by the first generation after establishment of anadromous stickleback in fresh water.

It would be advantageous for individuals of the two forms to mate assortatively because hybrids would probably have reduced fitness relative to the two parental forms. The life history switch from anadromous to resident may present the greatest obstacle for hybrid fitness given the large differences in migration behavior and physiological regulation (Johannesson 2001). Gene flow would be reduced if the two forms differed in the timing or place of reproduction or if courtship was disrupted by differences in morphology and behavior (McPhail 1994, McKinnon & Rundle 2002).

Imprinting of fry on their father's phenotype and choice of nest microhabitat might enable the rapid evolution of reproductive isolation in stickleback without necessitating a genetic correlation between female preference and male phenotype

(McPhail 1994). If double invasion facilitated by temporary barriers leads to species pairs in a couple of decades or less, "rapid speciation" may be a term that needs a temporal recalibration by at least two orders of magnitude. Perhaps the bulk of divergence in all types of stickleback species pairs (and species pairs of other fishes) occurred at the point of their formation, after which relative stasis dominated.

Independent Colonization Hypothesis

The species pair in Vitus Lake probably formed by independent colonization by resident stickleback from adjacent areas and by anadromous fish from the sea. Before Vitus Lake formed around 1900, nearby ponds, lakes and streams existed that must have contained resident and/or anadromous stickleback. As the drainage was rearranged with the retreating ice, these forms may have independently invaded the newly created Vitus Lake from these pre-existing waters. Similar independent colonizations could have given rise to the other species pairs.

The Tsiu River and wetlands has the greatest differentiation between the forms (Tables A – 5 & A – 6), and may be the oldest of the pairs given its greater distance from the glacier (Fig. A – 1). All anadromous fish were complete plate morphs, and nearly all resident fish were low plate morphs. This suggests that while gene flow between anadromous and resident forms may occur, it is sufficiently limited to consider them to be separate biological species. However, resident fish in Vitus Lake, Midtimber Lake and Creek 1 include low, partial and complete lateral plate morphs.

Phenotypic variation in the resident populations may reflect introgression by anadromous stickleback, stable polymorphism, or transient polymorphism during evolutionary transition. In the presence of gene flow, independent colonization allows reticulate evolution. Lateral plate polymorphism is controlled by a major locus and a series of modifiers. This polymorphism could be due to gene flow from the anadromous form preventing fixation at the low morph, which dominates in Alaskan lakes (Bell 2001, Bell et al. in press). Alternatively, partial and complete morph phenotypes may be present in the resident fish that colonize lakes during the independent colonization process. However, these results are also consistent with a recent origin from an anadromous ancestor, with the resident form in the process of evolving to a low-plated fish.

Differentiating Between Origin Hypotheses

Unfortunately, results to date do not allow us to reject any of the origin hypotheses. Divergence in the midst of gene flow is a pattern in the numerous independent cases of resident-anadromous species pairs (McKinnon & Rundle 2002). However, distinguishing between the double-invasion and independent colonization hypotheses may be possible by testing for genetic distance between members of a pair and adjacent populations that might have been the source of colonizers (see Taylor & McPhail). If the species pair is the result of a double invasion separated by a short-term block of the outlet, then two members of a pair should be derived in quick succession

from the same anadromous ancestor and might be nearly identical genetically, but genetically more distant from adjacent populations of the same life history. Conversely, if the pair is the result of independent colonization by pre-existing forms, then this should be reflected in a greater genetic distance between members of a pair than between adjacent populations of the same life history. The likelihood of double invasion as the origin of these species pairs might also be ruled out through more thorough study of the geomorphology.

Persistence of Species Pairs

Regardless of their origin, the persistence of these species pairs begs explanation. Whether these new pairs arose in allopatry, parapatry or sympatry, their maintenance or continued divergence takes place in sympatry, making speciation in this system a complex process. Their maintenance is likely due to reduced competition and predation in these lakes and streams due to the low density of fishes, allowing multiple variants to survive. The ecology of this region is dominated by abiotic factors such as temperature, suspended sediments, salinity, glacial surges and floods. There is no steady-state in these systems; disturbance is the norm.

Given that lake-dwelling species pairs of resident and anadromous stickleback are currently known from only a few locations, these species pairs may typically not persist for long after the lakes are formed. For example, it is possible that such species pairs formed numerous times along the periphery of the melting Laurentide and Cordilleran ice

sheets, but that within any particular lake gene flow between the forms eventually melded two into one (as may be happening in Midtimber Lake) or that competitive exclusion eventually caused the extinction of one. The Bering Glacier is a remnant of the Cordilleran ice sheet and an excellent analog to conditions at the end of the Pleistocene. The ecology of new lakes changes markedly as they evolve from ice-contact lakes with incredible sediment loads and periodic outburst floods to ice-free lakes with relatively stable hydrology. The dynamic nature and reduced competition and predation of ice-contact and nearby lakes may lead to the rapid formation of species pairs, but subsequent quiescence of the geomorphology and ecological stability may then lead to their dissolution.

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Table A.1. Six varieties of species pairs of threespine stickleback. [1]In Lakes Harutori, Akkeshi and Hyotan these two marine forms are also reproductively sympatric with a resident form (Mori 1990, Higuchi et al. 1996, Kitano et al. 2003), comprising the only known case of a species triplet, except possibly three morphs that specialize on different substrates and temperatures in an Icelandic geothermal pond (Jónsson & Ingólfsdóttir 2003).

Species Pair Type	Location
white marine/typical marine	Nova Scotia
Japan Pacific/Sea of Japan	coast of Hakkaido Island[1]
lake resident/stream resident	Vancouver & Graham Islands, British Columbia
Stream dwelling nuptially-red & nuptially-black	Olympic Peninsula, Washington
Lake dwelling limnetics & benthics	six lakes on three islands in the Strait of Georgia, British Columbia
resident freshwater/anadromous	throughout much of Holarctic range of species complex

Table A.2. Resident-anadromous species pairs discovered in the Bering Glacier drainage. The specificity of the GPS coordinates varies depending on the size of the lake or stream.

Location	GPS Coordinates	Habitat Type
Vitus Lake	60.1° N, 143.3° W	proglacial lake
Midtimber Lake	60.08° N, 143.35° W	Lake
Tsiu River/wetlands	60.08° N, 143.06° W	river-wetland complex
Creek 1	60.10482° N, 143.35008° W	creek draining into Vitus Lake
Creek 2	60.09770° N, 143.35117° W	creek draining into Vitus Lake
Unnamed Lake	60.10195° N, 143.55113° W	small lake upstream of Vitus Lake
Tashalich River	60.03905° N, 143.62566° W	river in the process of becoming a lake
Bering River side-lake	60.35771° N, 143.97263° W	small lake adjacent to large river

Table A.3. Morphological variables of 2002 samples of resident and anadromous stickleback in Vitus Lake, Alaska. Mean lengths and standard deviations are in mm. Reported here are the Mann-Whitney U statistics (because of small sample size) based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits, with the exception of SL, which was a direct comparison of raw SL values (see Methods). The significance of analyses remains the same when analyzed by one-way ANOVAs. Statistics are based on totals (left + right) of lateral plates and pelvic spines. Anadromous sample size for right pelvic spine length was 49 and second dorsal spine length 47. Plate morph 1 = low, 2 = partial, 3 = complete.

Variable Name	Resident FW mean, n=21 (sd)	Anadromous mean, n=50 (sd)	Mann-Whitney *U*	p
SL	37.3 (4.4)	62.9 (5.7)	0	< 0.001
Left pelvic spine length	4.8 (0.8)	8.6 (0.8)	73.0	< 0.001
Right pelvic spine length	4.8 (0.7)	8.6 (0.8)	-	-
Second dorsal spine length	3.2 (0.6)	6.0 (0.9)	117.0	< 0.001
Plate morph	2.0 (0.9)	3.0 (0)	200.0	< 0.001
Number left lateral plates	20.0 (13.0)	33.2 (0.8)	177.5	< 0.001
Number right lateral plates	20.0 (13.0)	33.2 (0.9)	-	-
Number gaps in left plates	0.4 (0.8)	0 (0)	-	-
Number gaps in right plates	0.2 (0.7)	0 (0)	-	-
Pelvic score left	4 (0)	4 (0)	-	-
Pelvic score right	4 (0)	4 (0)	-	-
Number gill rakers	20.8 (1.5)	23.3 (0.9)	54.5	< 0.001

Table A.4. Morphological variables of 2003 samples of resident and anadromous stickleback in Vitus Lake, Alaska. Mean lengths and standard deviations are in mm. Reported here are the univariate F's from a MANOVA based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits (see Methods). The univariate F for SL is the result of a one-way ANOVA. Fish were assigned as resident or anadromous by cluster analysis. Statistics are based on total (left + right) of pelvic spine lengths. Resident sample size for left pelvic spine length and dorsal spine length was 72. Anadromous sample size for dorsal spine length and number of dorsal fin rays was 46, left pelvic spine length 44, and right pelvic spine length 46. Plate morph 1 = low, 2 = partial, 3 = complete.

Variable Name	Resident FW mean, n=73 (sd)	Anadromous mean, n=47 (sd)	F	p
SL	42.0 (4.6)	61.0 (6.2)	370.23	< 0.001
Left pelvic spine length	5.1 (0.8)	8.7 (1.0)	108.07	< 0.001
Right pelvic spine length	5.2 (0.8)	8.7 (1.0)	-	-
Second dorsal spine length	3.5 (0.6)	6.0 (0.8)	76.52	< 0.001
Distance dorsal to hyperal	6.2 (0.8)	7.7 (1.0)	52.19	< 0.001
Dorsal fin base length	10.1 (1.2)	15.4 (2.0)	0.05	n.s.
Body depth	9.4 (1.2)	13.3 (1.7)	2.50	n.s.
Snout length	3.2 (0.5)	5.1 (0.7)	155.03	< 0.001
Orbit width	3.5 (0.3)	4.9 (0.5)	48.77	< 0.001
Left pectoral fin length	6.2 (0.9)	11.5 (1.6)	143.12	< 0.001
Plate morph	1.7 (0.8)	3.0 (0)	-	-
Number left lateral plates	17.4 (12.1)	33.0 (0.7)	-	-
Number right lateral plates	17.7 (12.1)	33.4 (0.8)	-	-
Number gaps in left plates	0.4 (0.7)	0 (0)	-	-

Number gaps in right plates	0.3 (0.7)	0 (0)	-	-
Pelvic score left	4.0 (0)	4.0 (0)	-	-
Pelvic score right	4.0 (0)	4.0 (0)	-	-
Number gill rakers	18.9 (1.0)	22.5 (1.2)	239.29	< 0.001
Number dorsal fin rays	11.0 (0.7)	11.7 (0.7)	29.78	< 0.001
Operculum width	3.1 (0.5)	5.9 (0.9)	227.30	< 0.001

Table A.5. Morphological variables of 2002 samples of resident and anadromous stickleback in the Tsiu River and wetlands, Alaska. Mean lengths and standard deviations are in mm. Reported here are the univariate F's from a MANOVA based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits (see Methods). The univariate F for SL is the result of a one-way ANOVA. Fish were assigned as resident or anadromous by cluster analysis. Statistics are based on total (left + right) of pelvic spines lengths. Resident sample size for left and right pelvic spine length was 200. Anadromous sample size for left and right pelvic spine length was 85 and left pectoral fin length was 84. Plate morph 1 = low, 2 = partial, 3 = complete.

Variable Name	Resident FW mean, n=201 (sd)	Anadromous mean, n=86 (sd)	univariate F	p
SL	45.3 (3.7)	62.4 (4.7)	1137.0	< 0.001
Left pelvic spine length	4.3 (0.5)	8.8 (0.9)	1625.81	< 0.001
Right pelvic spine length	4.5 (0.5)	8.8 (0.9)	-	-
Second dorsal spine length	3.2 (0.4)	6.2 (0.7)	935.03	< 0.001
Distance dorsal to hyperal	7.0 (0.9)	7.9 (0.9)	148.97	< 0.001
Dorsal fin base length	10.5 (1.1)	15.7 (1.6)	80.62	< 0.001
Body depth	10.1 (1.0)	12.9 (1.2)	15.71	< 0.001
Snout length	3.6 (0.4)	5.6 (0.7)	145.17	< 0.001
Orbit width	4.2 (0.4)	5.2 (0.4)	0.05	n.s.
Left pectoral fin length	5.8 (0.7)	12.1 (1.3)	1323.43	< 0.001
Plate morph	1.0 (0)	3.0 (0)	-	-
Number left lateral plates	5.3 (1.0)	33.1 (0.8)	-	-
Number right lateral plates	5.3 (1.8)	33.4 (0.8)	-	-
Number gaps in left plates	0 (0.1)	0 (0)	-	-
Number gaps in right plates	0 (0.2)	0 (0)	-	-

Pelvic score left	4 (0.1)	4 (0)	-	-
Pelvic score right	4 (0.1)	4 (0)	-	-
Number gill rakers	18.7 (0.9)	23.4 (1.2)	1143.54	< 0.001

Table A.6. Morphological variables of 2003 samples of resident and anadromous stickleback in the Tsiu River and wetlands, Alaska. Mean lengths and standard deviations are in mm. Reported here are the univariate F's from a MANOVA based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits (see Methods). The univariate F for SL is the result of a one-way ANOVA. Fish were assigned as resident or anadromous by cluster analysis. Statistics are based on total (left + right) of pelvic spine lengths. Resident sample size for left and right pelvic spine length was 204 and pectoral fin length 207. Anadromous sample size for left pelvic spine length was 197, right pelvic spine length 195, dorsal spine length 198, and pectoral fin length and number of gill rakers 199. Plate morph 1 = low, 2 = partial, 3 = complete.

Variable Name	Resident FW mean, n=208 (sd)	Anadromous mean, n=200 (sd)	univariate F	P
SL	46.4 (3.4)	65.3 (4.4)	2408.76	< 0.001
Left pelvic spine length	4.7 (0.5)	9.3 (0.8)	2815.08	< 0.001
Right pelvic spine length	4.7 (0.5)	9.4 (0.8)	-	-
Second dorsal spine length	3.3 (0.4)	6.8 (0.7)	1661.53	< 0.001
Distance dorsal to hyperal	7.3 (0.9)	8.1 (0.9)	421.58	< 0.001
Dorsal fin base length	10.7 (1.1)	16.7 (1.5)	121.36	< 0.001
Body depth	10.6 (0.9)	14.2 (1.2)	0.22	n.s.
Snout length	3.6 (0.4)	5.4 (0.5)	539.31	< 0.001
Orbit width	4.1 (0.4)	5.2 (0.3)	36.10	< 0.001
Left pectoral fin length	6.2 (0.6)	12.9 (1.0)	4027.15	< 0.001
Plate morph	1.0 (0.2)	3.0 (0)	-	-
Number left lateral plates	5.3 (2.5)	33.5 (0.7)	-	-
Number right lateral plates	5.3 (2.5)	33.5 (0.7)	-	-
Number gaps in left plates	0 (0.2)	0 (0)	-	-

Number gaps in right plates	0 (0.2)	0 (0)	-	-
Pelvic score left	4.0 (0)	4.0 (0)	-	-
Pelvic score right	4.0 (0)	4.0 (0)	-	-
Number gill rakers	18.9 (0.9)	23.3 (1.0)	1785.73	< 0.001
Number of dorsal fin rays	10.3 (0.7)	11.7 (0.7)	300.50	< 0.001
Operculum width	3.8 (0.5)	7.6 (0.7)	1817.19	< 0.001

Table A.7. Morphological variables of 2003 samples of resident and anadromous stickleback in Creek 1, near the Bering Glacier, Alaska. Mean lengths and standard deviations are in mm. Reported here are the Mann-Whitney U statistics (because of small sample size) based on z-transformed meristic traits and adjusted residuals of z-transformed morphometric traits, with the exception of SL, which was a direct comparison of raw SL values (see Methods). The significance of analyses remains the same when analyzed by one-way ANOVAs. Statistics are based on totals (left + right) of lateral plates and pelvic spines. Anadromous sample size for left and right pelvic spine and dorsal spine was 33. Plate morph 1 = low, 2 = partial, 3 = complete.

Variable Name	Resident FW mean, n=14 (sd)	Anadromous mean, n=34 (sd)	Mann-Whitney U	p
SL	44.2 (5.6)	64.5 (5.7)	8.0	< 0.001
Left pelvic spine length	5.3 (0.7)	9.3 (1.0)	0	< 0.001
Right pelvic spine length	5.5 (0.8)	9.3 (1.1)	-	-
Second dorsal spine length	3.5 (0.5)	6.4 (0.9)	0	< 0.001
Distance dorsal to hyperal	6.3 (0.8)	8.2 (1.0)	147	.039
Dorsal fin base length	10.5 (1.7)	16.1 (1.7)	203.0	n.s.
Body depth	9.8 (1.1)	13.9 (1.4)	183.0	n.s.
Snout length	3.6 (0.4)	5.3 (0.7)	98.0	.001
Orbit width	3.8 (0.4)	5.1 (0.5)	146.0	.037
Left pectoral fin length	6.2 (0.8)	12.3 (1.3)	0	< 0.001
Plate morph	1.9 (0.9)	3.0 (0)	85.0	< 0.001
Number left lateral plates	16.5 (12.6)	33.9 (0.7)	23.0	< 0.001
Number right lateral plates	16.9 (12.8)	33.6 (0.8)	-	-
Number gaps in left plates	0.4 (1.1)	0 (0)	-	-
Number gaps in right plates	0.2 (0.6)	0 (0)	-	-

Pelvic score left	4.0 (0)	4.0 (0)	-	-
Pelvic score right	4.0 (0)	4.0 (0)	-	-
Number gill rakers	19.2 (1.1)	22.7 (1.2)	5.5	< 0.001
Number of dorsal fin rays	11.3 (0.8)	11.5 (0.7)	200.0	n.s.
Operculum width	3.6 (0.8)	7.5 (0.7)	0	< 0.001

Table A.8. Results of MANOVAs comparing the morphology of resident fish from Vitus Lake, Midtimber Lake and the Tsiu River and wetlands.

Comparison	Trait	Multivariate F	Univariate F	df	p
Vitus-Midtimber	"defense" traits	26.38		5, 137	< 0.001
	Dorsal spine length		4.60	1, 141	0.034
	Body depth		73.39	1, 141	< 0.001
	Total pelvic spine length		1.95	1, 141	n.s.
	Distance dorsal-hyperal		54.77	1, 141	< 0.001
	Pectoral fin width		12.09	1, 141	< 0.001
	"trophic" traits	15.48		5, 140	< 0.001
	Operculum width		0.27	1, 144	n.s.
	Snout length		38.61	1, 144	< 0.001
	Orbit width		33.88	1, 144	< 0.001
	Number gill rakers		26.46	1, 144	< 0.001
	Number dorsal fin rays		4.97	1, 144	0.019
Vitus-Tsiu	"defense" traits	75.11		5, 135	< 0.001
	Dorsal spine length		48.00	1, 139	< 0.001
	Body depth		80.21	1, 139	< 0.001
	Total pelvic spine length		72.05	1, 139	< 0.001
	Distance dorsal-hyperal		8.78	1, 139	0.004
	Pectoral fin width		266.24	1, 139	< 0.001
	"trophic" traits	51.50		5, 140	< 0.001
	Operculum width		87.94	1, 144	< 0.001
	Snout length		189.16	1, 144	< 0.001
	Orbit width		68.13	1, 144	< 0.001
	Number gill rakers		6.55	1, 144	0.012
	Number dorsal fin rays		6.17	1, 144	0.014
Midtimber-Tsiu	"defense" traits	6.05		5, 136	< 0.001
	Dorsal spine length		5.99	1, 140	0.016

Comparison	Trait	Multivariate F	Univariate F	df	p
	Body depth		2.29	1, 140	n.s.
	Total pelvic spine length		11.27	1, 140	0.001
	Distance dorsal-hyperal		11.49	1, 140	0.001
	Pectoral fin width		10.08	1, 140	0.002
	"trophic" traits	19.93		5, 140	< 0.001
	Operculum width		14.00	1, 144	< 0.001
	Snout length		12.46	1, 144	0.001
	Orbit width		4.44	1, 144	0.037
	Number gill rakers		48.19	1, 144	< 0.001
	Number dorsal fin rays		22.47	1, 144	< 0.001

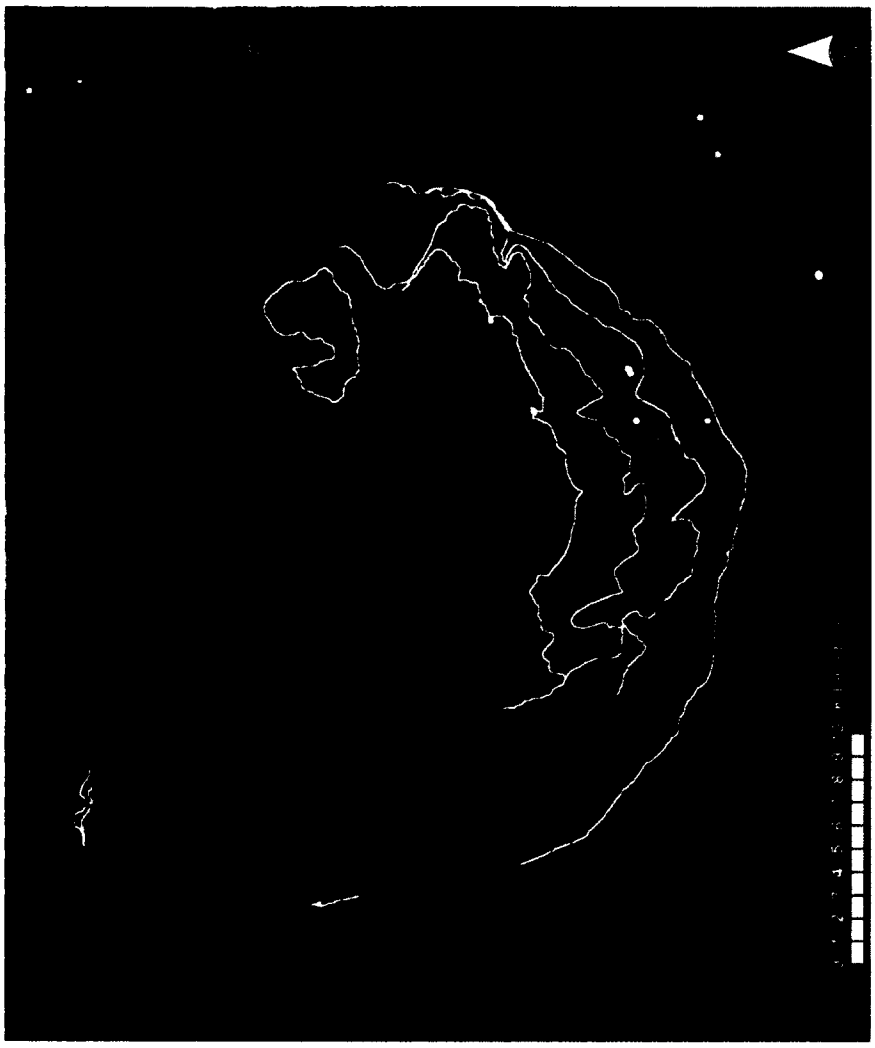


Figure A.1. Landsat imagery of the Bering Glacier region showing the location of eight stickleback species pairs (Vitus Lake, Midtimber Lake, Tsiu River, Creeks 1 and 2, Unnamed Lake, Tashalich River, and Bering River side-lake) and other landscape features discussed in text. The location of sampling points with a species pair of stickleback is shown for samples collected in 2002, 2003 and 2004. Image was captured September 10, 2001. The position of the ice is shown at Little Ice Age maximum of 1900 as well as at maximum 1967 surge position, before the most recent surge in 1993, and at maximum recent surge position in 1995.

Appendix B IACUC Approvals 2001, 2004, and 2007

University of Alaska Anchorage
University of Alaska Anchorage
Institutional Animal Care and Use Committee

Memo

To: Frank von Hippel

From: Jennifer Burns, Chair, IACUC

CC: Rick Bernhardt

Date: 2001

Re: IACUC Approval 2001vonHi1

Frank,

Your research project ' Sticklebacks in Lab and Field' was approved by the UAA IACUC committee on 5/2/2001, and is good for a period of three years. You must you submit annual reports to the IACUC each year. Currently, you are in good standing with this protocol.

Jennifer M Burns

Jennifer Burns, IACUC chair

University of Alaska Anchorage
Institutional Animal Care and Use Committee

Memo

To: Dr. Frank von Hippel

From: Jennifer Burns, Chair, IACUC

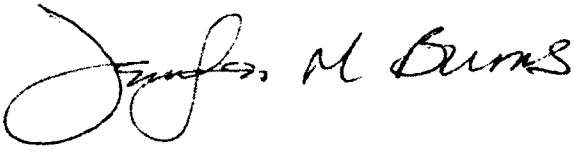
Date: May 10, 2004

Re: IACUC protocols "Behavior, evolution, biogeography and conservation of Alaskan freshwater fishes"

Frank –

The Institutional Animal Care and Use Committee at the University of Alaska Anchorage has approved your protocols "Behavior, evolution, biogeography and conservation of Alaskan freshwater fishes".

Your approval is good for a period of 3 years, and will expire May 10, 2007. You are required to submit an annual report of your activities (form available at http://www.uaa.alaska.edu/iacuc/protocols.html) by May 1st of each year. We would also like to remind you that any changes in personnel or protocols must also be submitted to the committee. Thank you for your support of animal care guidelines. We hope that your research goes well.



Jennifer Burns, Chair IACUC

University of Alaska Anchorage
Institutional Animal Care and Use Committee

Memo

To: Dr. Frank von Hippel

From: Eric S. Murphy, Chair, IACUC

Date: 4/5/07

Re: IACUC protocol 2007vonhi1

The Institutional Animal Care and Use Committee at the University of Alaska Anchorage met on March 9, 2007 and reviewed the mandatory rewrite of your protocol "Behavior, evolution, biogeography, and conservation of Alaskan freshwater fishes", that you submitted to us on March 5, 2007. After receiving the documentation that we requested, UAA's IACUC is pleased to approve your protocol. Please note that this approval is contingent upon your compliance with all relevant University, city, state, and federal regulations, and requires that you possess all relevant permits before work is initiated. Your protocol ID number is 2007vonhi1. Your approval is good for a period of 3 years, and will expire on 4/5/2010. You are required to submit an annual report of your activities prior to 4/5 in each of the next two years. This form is available at http://www.uaa.alaska.edu/iacuc/protocols.html.

We remind you that all changes in personnel and animal handling protocols must be submitted to the committee prior to such changes taking place. In addition, should you experience any unexpected animal mortalities, illnesses, or injury (to animals or personnel involved with the project), you are required to report such to the IACUC immediately.

Thank you for your support of animal care guidelines. We hope that your research goes well.

Eric S. Murphy, Chair

UAA IACUC